

# Elevated mutation and selection in wild emmer wheat in response to 28 years of global warming

Yong-Bi Fu, Gregory W. Peterson, Carolee Horbach, David J. Konkin, Avigdor Beiles and Eviatar Nevo

## Supporting Information Appendix

- A. Supplemental materials and methods
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### A. Supplemental materials and methods

This section consists of 10 components describing the study materials, the procedures used to perform various sequence and genetic analyses, and data and code availability. For ease of understanding the complex and lengthy analyses, a flowchart was generated to describe the major steps from population sampling to ontological analysis of deleterious genes (Fig. S1).

#### A1. Study materials and DNA extraction

Ten populations of wild emmer wheat (WEW) from across Israel, West Bank, and Golan Heights (see Nevo et al. 2012: Fig. 1A) were selected and the seeds of selected plants up to 100 meters apart were collected in 1980 and again in 2008 by Professor Eviatar Nevo. These collection sites remained largely intact without any habitat disturbances over the 28 years. The climate data in Israel from 1980 to 2010 were also obtained from Israel Meteorological Service and Prof. Yair Goldreich's report (No. 8-814) to the Ministry of Environmental Quality in 2010 on climate change trend analysis. Previous analyses of these climate data revealed a rising temperature and declining rainfall from 1980 to 2010 in Israel (see Figs. S4 to S6 of Nevo et al. (2012)). These patterns of climate change were consistent with the trends for the rising temperature and declining rainfall in Israel from 1980 to 2009, as reported recently from the World Bank website [Tradingeconomics.com](http://Tradingeconomics.com) (Fig. 1C and Fig. 1D). To have sufficient seeds for future studies, the collected seeds for each plant were increased once in a greenhouse at the University of Haifa and the increased seeds were maintained separately for each plant. The increased seeds were sent to the Saskatoon Research and Development Centre and planted in a greenhouse under 16 hours daylight. Young leaves were collected prior to flowering from each plant and freeze-dried for storage prior to DNA isolation.

DNA was extracted from 91 samples of WEW from the 1980 collection and 95 samples from the 2008 collection, representing 10 populations (Table S1). Ten mg of freeze dried tissue per sample was ground in a 2-ml microcentrifuge tube with three 3-mm glass beads in a mixer mill and applied to the NucleoSpin Plant II purification kit (Macherey-Nagel). Extracted DNA was eluted into 50 to 100  $\mu$ l of 5 mM Tris pH 8.5 and quantified using the Quant-iT Picogreen dsDNA Assay Kit (Thermo Fisher Scientific - Invitrogen). DNA was diluted to 30 ng/ $\mu$ l with 5 mM Tris pH 8.5.

## A2. Exome capture sequencing

Exome capture libraries were prepared using the Kapa HyperPlus DNA library preparation kit (Roche – Kapa Biosystems) to fragment and size-select the genomic DNA samples (Henry et al. 2014). Protocol KR1145, v4.17, was followed. Briefly, 1 µg of each genomic DNA sample was fragmented for 15 min at 37°C in a C1000 thermocycler (Bio-Rad Canada) with a lid temperature of 50°C. Samples were end-repaired and A-tailed for 30 min at 65°C with a lid temperature of 80°C in the same thermocycler followed by the ligation of 50 µM NEXTflex96 DNA barcoded Illumina adapters (Perkin Elmer – BIOO Scientific) at 20°C for 15 min in a Bio-Rad PTC200 thermocycler with a lid temperature of 30°C. Forty-eight unique NEXTflex96 adapters were selected. The ligated samples were cleaned using Kapa Pure Beads (Roche – Kapa Biosystems) following the HyperPlus protocol and eluted in 50 µl of 10 mM Tris-HCl, pH 8.0. Size selection was done using Kapa Pure Beads at 0.6x and 0.8x sample volume following the HyperPlus protocol to select for fragments between 300 bp and 700 bp with modal target of 450 bp (≈350 bp of genomic DNA plus adapters) and eluted in 20 µl of 10 mM Tris-HCl, pH 8. The samples were amplified for 5 to 7 cycles using PCR according to the HyperPlus kit. The final PCR produced was cleaned using 1x sample volume of Kapa Pure Beads and eluted in 50 µl of BPC Grade water (Sigma-Aldrich Canada).

Size selected WEW fragments were captured in pools of 48 using a custom wheat bait library (SeqCap EZ Design 160318\_Wheat\_Tae\_Red\_EZ\_HX1, Roche – NimbleGen) following manufacturer's instructions. This library was expected to capture 152,156 exome regions across the WEW genome (Jordan et al. 2015). Sequencing of the multiplexed libraries was carried out on an Illumina HiSeq 2500, SBS Version 4, at the National Research Council of Canada, Saskatoon, SK, Canada, in two lanes running 2x125-cycle paired-end sequence reads.

## A3. Raw sequence processing and SNP calling

Exome capture sequence data were received as two demultiplexed FASTQ files per sample; one file for each end of the sample's library fragments. A representative sample of fragments were assessed with FastQC (Babraham Bioinformatics; <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) to determine overall sequencing quality. Samples were trimmed with Trimmomatic v0.32 (Bolger et al. 2014) to remove residual Illumina adapter sequence, trim low quality sequence below an average Phred score of 24 over a 10 base window, and any sequence shorter than 80 bases. FastQC was again used on a representative sample to verify that the Illumina adapter sequences were removed.

The reference genome sequence, WEWseq\_v1 from *Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell. “Zavitan” (Avni et al. 2017), or “wew”, was modified for use with the WEW samples. The WEW reference chromosomes were split in half using a custom Perl script (halve\_wew\_chromos.pl) to reduce the individual chromosome sequence length below the 2<sup>39</sup>-1 base (≈536 Mb) limit of Samtools BAM indexing and the split genome sequence was used as a reference sequence for the WEW analysis. Each sample was aligned against the reference genome using the Burrow-Wheeler Aligner v1.7 (Li and Durbin 2010) BWA-MEM algorithm. The resulting bam files were processed with the Genome Analysis Toolkit (GATK; Van der Auwera et al. 2013) v4.0.1.0 tools to remove PCR duplicates (MarkDuplicates) and to apply base quality score recalibration (BQSR; BaseRecalibrator and ApplyBQSR). Two WEW samples 2008-07-16-ISR and 2008-08-09-ISR were removed from further analysis due to the low number of sequence reads relative to the rest of the population.

### **A3a. Ancestral genome**

An ancestral genome was predicted for WEW by aligning *Triticum urartu* Thumanjan *ex* Gandilyan (TU) (Ensembl Plants assembly ASM34745v1; Ling et al. 2018) against the WEWseq\_v1 reference sequence. The lastz\_32 method (Harris 2007) was used to align the TU sequences against the WEW reference genome and convert the alignment into maf files for each WEW chromosome and set of unassigned scaffolds. The individual "ancestral" chromosomes were split in the same manner as the WEW reference genome, for downstream applications that were reliant upon htlib tools (e.g. Samtools; Li et al. 2009), concatenated into a single ancestral genome, and indexed for use with Samtools and related applications.

### **A3b. SNP calling and annotation**

Single nucleotide polymorphisms (SNPs) were called based on 91 and 93 cleaned bam files using ANGSD (Korneliussen et al. 2014) for the samples collected in 1980 and in 2008, respectively. The half chromosomes were recombined in the resulting variant call format (VCF) files using custom Perl scripts (vcf\_coord\_emmer.pl). SNP annotations were conducted using stand-alone Ensembl Variant Effect Predictor (VEP) (McLaren et al. 2016; Naithani et al. 2017) based on the ANGSD VCF output. A SIFT (Sorting Intolerant from Tolerant) database was specifically generated for this WEW study following the procedures used to create your own SIFT prediction database of Vaser et al. (2015). SIFT analysis was made separately from Ensembl VEP to generate SIFT scores for detected SNPs. A custom Perl script was used to filter and identify deleterious SNPs (dSNPs) for different classes of sequence such as 3'UTR, 5'UTR, downstream, upstream, nonsynonymous and synonymous. Related genes for different classes of SNPs were generated for further analyses. The SNP annotation was done separately for each sampling year.

### **A3c. GERP++ Rejected Substitution scores**

To enhance the detection of dSNPs, we also generated GERP++ Rejected Substitution (RS) scores, besides SIFT scores. GERP++ (Davydov et al. 2010) measures the constraint from substitution of a locus by generating an RS score. Gerpcol, specifically, estimates constraint for each column of an alignment of several genomes of increasing taxonomic distance. Multiple whole-genome sequence alignment was carried out for 12 species against emmer (Table S3). Due to the large overall genome size and large individual chromosome size, barley and urartu were aligned to the individual WEW chromosomes using Mugsy v1.2.3 (Angiuoli and Salzberg, 2011) and the resulting alignments were in multiple alignment format (maf). The remaining 10 genomes were aligned with the large-scale genome alignment tool (LASTZ) (Harris 2007) and converted to maf using: axtChain, chainNet, netSyntenic, netToAxt, and axtToMaf (UCSC Genome Browser Toolkit, Anaconda distribution) against the WEW chromosomes.

For each emmer chromosome-crop genome combination, single\_cov2.v11 from the Multiz package (Blanchette et al. 2004) was used to remove any low-scoring alignments where there was overlap in alignments against each emmer chromosome to generate a single coverage alignment across each emmer chromosome. Roast (Hou and Riemer 2008) was used to find orthologous alignments for each emmer chromosome across all 12 crop genomes and to generate a single maf file for each emmer chromosome. Phylogenetic tree and neutral branch length (estimated from fourfold degenerate sites) analyses were made using PhastCons (msa\_view and phyloFit; Siepel et al. 2005) and was used to quantify the constraint intensity at every position in the emmer genome. Emmer genome sequences were eliminated during the site-specific observed

estimates (RS scores) to eliminate the confounding influence of deleterious derived alleles segregating in emmer samples present in the reference sequence.

### **A3d. Identifying dSNPs**

Amino acid substitutions and their effects on protein function were predicted with the SIFT algorithm. Nonsynonymous mutations with SIFT scores  $<0.05$  were defined as putative deleterious mutations. SIFT ( $<0.05$ ) and GERP++ RS ( $>0$ ) annotations were combined to identify the deleterious mutations in constrained portions of the genome. These deleterious mutations were used to calculate the WEW mutational burden to characterize dSNPs. We applied TU sequences as an ancestral genome to identify ancient alleles. At a given position, if a TU reference allele matched the WEW reference allele, the allele in emmer was categorized as an ancient allele. If an emmer allele was different from the TU allele, the emmer allele was defined as a derived allele.

The analysis of dSNPs was done with respect to population, climate group, and year. For each population in each sampling year, SNP calling was made following **A3b** steps from all the cleaned bam files of the population and deleterious SNPs were identified from the VCF file based on the global list of deleterious SNPs that was generated from SIFT and GERP++ RS scores in the corresponding sampling year. Similarly, for a climate-specific group (see Fig. S1a), its bam files consisted of those from grouped populations. For example, the climate-specific group temp1 had 26 and 25 bam files from populations 1, 2 and 10 for two sampling years, respectively. SNP calling was made following **A3b** steps from its bam files, and dSNPs were identified similarly to the population analysis. These dSNP files with respect to population, group and year formed the basis for many of the population genetic analyses below.

### **A4. Measuring mutational burden.**

With identified SNP information, ANGSD was run for genotyping and obtaining derived allelic frequency for WEW samples. Mutational burden for individual samples was calculated from sample genotype data based on the numbers of derived deleterious alleles present in three models: homozygous-mutational burden, heterozygous-mutational burden, and total mutational burden (Wang et al. 2017). The homozygous-mutational burden is the number of derived deleterious alleles in the homozygous state. The heterozygous-mutational burden is the number of derived deleterious alleles existing in the heterozygous state. The total mutational burden is the number of derived deleterious alleles existing in an accession ( $2 \times$  homozygous-mutational burden + heterozygous-mutational burden). A population mutational burden was calculated based on mean of individual total mutational burden. We also generated a population weighted RS burden by weighting RS score of each dSNP with its population allelic frequency and averaging weighted RS across all the dSNPs. A higher weighted RS score means more mutational burden for the population. Note that population allelic frequencies were obtained using ANGSD with the detected SNP sites. The analysis of mutational burden was done with respect to population, group and year.

### **A4a. Analyzing allelic frequencies of dSNPs**

Based on ANGSD-generated maf files, allelic frequency distributions were analyzed for dSNPs identified with SIFT and GERP++ RS scores in each sampling year. Analysis was also done for dSNPs with two extreme frequencies, and fixed dSNPs were identified in samples of each sampling year.

### **A5. Identifying selective sweeps.**

Two approaches were applied to identify selective sweeps across the WEW genome. RAiSD (Raised Accuracy in Sweep Detection) was a fast, parameter-free detection system using multiple signatures of a selective sweep via the enumeration of SNP vectors (Alachiotis and Pavlidis 2018). Based on an ANGSD-generated VCF file, MuStat was generated for each sliding-window of default size across each chromosome and outliers with 9, 15 and 20 standard deviations were used to define tentative selective sweep regions.

The neutrality test statistic Tajima's  $D$ , although less accurate due to compounding effects of demography and other factors (Korneliussen et al. 2013), was also applied to acquire indirect selection signal. It was calculated using ANGSD with an empirical Bayes approach (Korneliussen et al. 2013). A global site frequency spectrum (SFS) was estimated and the posterior sample allele frequencies was calculated using the global SFS as a prior. The Tajima's  $D$  statistics were summarized across the genome using 50-kb non-overlapping sliding windows with steps of 10-kb. The outlier sliding-window with a negative Tajima's  $D$  estimate smaller than their 3 standard deviations were considered to carry a selection signal. Additional analysis was also made to obtain Tajima's  $D$  statistics for different classes of sequences representing 3'UTR, 5'UTR, downstream, upstream, nonsynonymous and synonymous substitution regions and types. The analysis of selective sweep was done with respect to population, climate group, and year.

### **A6. Estimating nucleotide diversity**

Watterson's  $\theta$  and Tajima's nucleotide diversity  $\pi$  statistics were calculated using ANGSD with an empirical Bayes approach (Korneliussen et al. 2014). It was obtained using the same approach as for calculating Tajima's  $D$  statistics. These diversity statistics were summarized for each chromosome and whole genome using 50-kb non-overlapping sliding windows with steps of 10-kb. This was done with respect to population, group and year. To understand the changes in nucleotide diversity, derived allelic frequency distribution was also inferred from the population-based maf file for all the detected SNPs with respect to population, group and year.

Estimation of per-individual inbreeding coefficients ( $F_{IS}$ ) under a probabilistic framework was also made within each population using ngsF (Vieira et al. 2013) with initial values of  $F_{IS}$  set to be uniform at 0.01 with an epsilon value of  $1e^{-5}$ . The estimation was also extended to samples for group and year, but was less reliable with pooled samples. Efforts were also made to analyze genetic differentiation ( $F_{ST}$ ) over the 28 years with respect to population, group and year using ngsTools/ANGSD (Fumagalli et al. 2014).

### **A7. Inferring adaptive mutations**

Adaptive mutations in emmer were inferred following polyDFE (Tataru et al. 2017) with the proportion of adaptive substitutions (i.e., those with selection coefficient greater than zero),  $\alpha$ -dfe, which was defined as the ratio of the estimated adaptive substitutions over the observed selected divergence counts (Loewe et al. 2006). For samples of each population, group or year, SFS data were generated based on synonymous and non-synonymous SNPs using ANGSD for the whole genome. Total sequence length of the selected regions (TLs) was first estimated by the product of the non-synonymous SNP count times 110 bp/SNP; the latter was roughly estimated by the total exome genome length divided by the total SNPs detected in this

study. Total sequence length of the neutral regions was estimated following the proportionality principle with 1/3 of the TLs (Tataru et al. 2017). Such estimation of the sequence length may be conservative and could scale up the alpha-dfe estimation. Three models (A, C and D) using the `-w` option were examined and the resulting alpha-dfe estimates were compared. Final analysis was done with Model A, as it was the most stable model with convergence. Each polyDFE analysis was done with 30 bootstrapped datasets. The analysis of adaptive mutation was done with respect to population and group, but not for year, as polyDFE version 2.0 is limited to a population or group of diploid sample size 50.

#### **A8. Gene ontology (GO) analysis of dSNPs and selective genes**

Genes or canonical transcripts associated with dSNPs identified in the two sampling years were extracted from VEP file. The extracted genes were analyzed by Blast2GO Pro v.5.2.5 (Conesa and Götz 2008) using the Gene Ontology Annotation workflow (blast, mapping, and annotation) and Enrichment Analysis (Fisher Exact Test). Non-redundant GO term sets were visualized using REVIGO (Supek et al. 2011) with treemaps and tag clouds to assist biological interpretation. These GO analyses were also conducted for genes associated with fixed dSNPs and with respect to common and unique genes in each sampling year.

Efforts were also made to perform a comparative GO analysis of the selective genes in the selective genomic regions identified by RAI<sub>SD</sub>. First, the selective genomic regions were identified with RAI<sub>SD</sub> MuStat estimates of 20 standard deviations or larger. Second, HOMER pipeline (Heinz et al. 2010) was applied to extract non-redundant genes from the selective regions. Third, the extracted genes were subject to the GO analysis as for dSNPs.

**A9. Variation analysis for climate-specific groups:** We evaluated the impacts of rainfall and temperature on genetic responses (or estimates of genetic parameters) in wild emmer wheat by grouping the 10 populations with climate factor profiles to three rainfall and three temperature population groups (Fig. S1a), estimating 14 genetic parameters in each group, and testing the differences in genetic estimates among climate-specific groups by Kruskal-Wallis one-way ANOVA (Kruskal and Wallis 1952). The non-parametric test was applied in this study, mainly considering the unbalance in sampling and possible complication of tetraploid WEW for genetic inference. Note that our individual sampling was relatively small for genetic inference with roughly nine individual plants per population, but it was adequate for genetic comparison across the genome (see the discussion in Chapter 10 of the book *Molecular Evolutionary Genetics*; Nei (1987)). The test was conducted using R basic package (R Development Core Team, 2008). For each parameter of interest (e.g., individual total load), generally, there were 24 Kruskal-Wallis rank sum tests with respect to population (2), climate group (4) and year (18). These tests essentially allow for an assessment of significant differences between or among population or group medians of a genetic estimate. All figures for this paper with bar and Manhattan plots or histograms were generated using custom R scripts based on existing R packages.

Additional efforts were also made to evaluate the extent of selective sweeps identified across the 14 WEW chromosomes in each climate-specific group and to compare allele frequency distributions between two sampling years for all detected SNPs and for all dSNPs in six climate-specific groups. These analyses allowed for better understanding of genetic changes and selection with respect to climate-specific group.

## **A10. Data and code availability**

Acquired exome capture data (Table S2) were deposited in NCBI's SRA database under BioProject ID: PRJNA507456. Three supplemental output data sets (wild-emmer-GERP\_RS-larger-than-zero.txt.gz, wild-emmer-exomecapture-e1980-19672del-alleles.txt, and wild-emmer-exomecapture-e2008-18627del-alleles.txt) are deposited into Figshare (<https://doi.org/10.6084/m9.figshare.7107443>). Custom Perl, Shell and R scripts or related pipelines that we generated for the bioinformatics analyses of WEW exome capture data will be available upon request to the senior author.

## **B. References for materials and methods**

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## C: Grouping of supplemental tables and figures

### C1. Material, sequencing, SNP identification and annotation:

**Table S1.** List of emmer wheat sample location, geographical information, label and sampling year

**Table S2a.** Summary of exome capture sequence reads for each sequenced sample of emmer wheat collected in 1980.

**Table S2b.** Summary of exome capture sequence reads for each sequenced sample of emmer wheat collected in 2008.

**Table S3.** List of plant species used to generate GERP++ RS scores for identification of deleterious SNPs in wild emmer wheat samples.

**Table S4.** SNP discovery and annotation in the samples of wild emmer wheat collected in 1980 and in 2008.

**Fig. S1.** A flowchart showing the overall procedures with eight major steps from population sampling (A1) to ontological analysis of deleterious genes (A8).



## C2. Nucleotide diversity

**Table S5.** Summary statistics and test significance for the mean Watterson's theta estimates per chromosome ( $aWc$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S6.** Summary statistics and test significance for the mean nucleotide diversity  $\pi$  estimates per chromosome ( $aPc$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S7.** Summary statistics and test significance for mean individual inbreeding coefficient ( $Fis$ ) and population differentiation over years ( $Fst$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S2.** Nucleotide diversity estimated with Watterson's Theta  $\theta$  (top panel) and Tajima's  $\pi$  (bottom panel) across 14 chromosomes in individual samples of wild emmer wheat collected in 1980 and in 2008.

## C3. Selective sweep

**Table S8.** Summary statistics and test significance for the proportions of significant sliding windows per chromosome with 9 standard deviations of  $RAiSD$  MuStat ( $pSWC9$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S9.** Summary statistics and test significance for the proportions of significant sliding windows per chromosome with 15 standard deviations of  $RAiSD$  MuStat ( $pSWC15$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S10.** Summary statistics and test significance for the proportions of sliding windows per chromosome with positive Tajima's D statistics greater than 3 standard deviations ( $PSW>3D$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S11.** Summary statistics and test significance for the proportions of sliding windows per chromosome with negative Tajima's D statistics ( $PSW<0$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S3a.** Selective sweeps across the first 7 wild emmer wheat chromosomes (chr1A to chr4A) identified by  $RAiSD$  mu statistics (MuStat) from exome capture data in samples of wild emmer wheat collected in 1980 and in 2008.

**Fig. S3b.** Selective sweeps across the second 7 wild emmer wheat chromosomes (chr4B to chr7B) identified by  $RAiSD$  mu statistics (MuStat) from exome capture data in samples of wild emmer wheat collected in 1980 and in 2008.

**Fig. S4.** Tajima's D statistics estimated across 14 chromosomes in the wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S5.** Tajima's D statistics obtained in the samples of wild emmer wheat collected in 1980 and in 2008, with respect to different classes of sequence.

## C4. Deleterious mutation

**Table S12.** Summary counts of total SNPs, total deleterious SNPs (dSNPs) and proportional deleterious SNPs (PDS) identified in wild emmer wheat collected in 1980 and in 2008, with respect to population, climate group and year.

**Table S13.** Summary statistics and test significance for the mean counts of deleterious SNPs per chromosome ( $delSNPc$ ) in wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S6a.** The distribution of deleterious SNPs (dSNPs) with non-overlapping sliding windows of 1 million base pairs across the first 7 chromosomes (Chr1A-Chr4A) for the 1980 and 2008 samples, highlighted in blue and red circles, respectively.

**Fig. S6b.** The distribution of deleterious SNPs (dSNPs) with non-overlapping sliding windows of 1 million base pairs across the second 7 chromosomes (Chr4B-Chr7B) for the 1980 and 2008 samples, highlighted in blue and red circles, respectively.

**Fig. S7.** Allele frequency (AF) distributions for deleterious SNPs (dSNPs) identified based on GERP++ RS scores determined from 12 plant species in individual samples of wild emmer wheat collected in 1980 and in 2008.

**Fig. S8.** Comparative counts of deleterious SNPs (dSNPs) with two extreme allelic frequencies from exome capture data in the samples of wild emmer wheat collected in 1980 and in 2008.

**Fig. S9.** Comparative allele frequency distributions for all the deleterious SNPs (dSNPs) detected in each population of wild emmer wheat between the collections in 1980 and in 2008.

**Fig. S10.** Comparative allele frequency distributions for all the detected SNPs that were shared in each population of wild emmer wheat between the collections in 1980 and in 2008.

### **C5. Adaptive mutation**

**Table S14.** Summary statistics and test significance for the estimates of dfe-alpha by polyDFE (in 30 bootstrapped samples) in wild emmer wheat samples collected in 1980 and in 2008.

### **C6. Mutational burden**

**Table S15.** Summary statistics and test significance for individual heterozygous load estimates per deleterious locus (Het load) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S16.** Summary statistics and test significance for individual homozygous load estimates per deleterious locus (Hom load) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S17.** Summary statistics and test significance for individual total load estimates per deleterious locus (TA load) in wild emmer wheat samples collected in 1980 and in 2008.

**Table S18.** Summary statistics and test significance for RS-based population load estimates (PopRS load) in wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S11.** Mutational burdens estimated for individual samples of wild emmer wheat collected in 1980 and in 2008.

**Fig. S12.** Distribution of GERP++ RS scores based on 12 species for SNPs identified from exome capture data in the combined samples of wild emmer wheat collected in 1980 and in 2008.

**Fig. S13.** Distributions of RS and weighted RS scores for deleterious SNPs (dSNPs) in 1980 and 2008 wild emmer wheat samples.

### **C7. Gene ontology analysis**

**Fig. S14a.** REVIGO gene ontology cluster representations showing the shared (in blue) and unique (in brown) biological processes associated with 317 and 272 GO terms extracted from 9,616 and 9,091 deleterious genes that were unique to the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively.

**Fig. S14b.** An illustration of 28 and 18 unique REVIGO gene ontology biological processes associated with 317 and 272 GO terms extracted from 9,616 and 9,091 deleterious genes that were unique to the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively.

**Fig. S15.** REVIGO gene ontology treemaps showing the biological processes associated with 3 and 14 GO terms extracted from 93 and 93 fixed deleterious genes that were unique to the

samples of wild emmer wheat collected in 1980 (top panel) or in 2008 (bottom panel), respectively.

**Fig. S16.** Tag clouds showing keywords that correlate with the values based on 1,044 and 1,022 GO terms for all the deleterious genes detected in the samples of wild emmer wheat collected in 1980 (top panel) and in 2008 (bottom panel), respectively.

**Fig. S17a.** Illustration of the shared (in blue) and unique (in brown) REVIGO gene ontology cluster representations for biological processes associated with 159 and 336 GO terms extracted from 66 and 80 non-redundant genes that were identified by RAI<sub>i</sub>SD MuStat estimates of 20 standard deviations in the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively.

**Fig. S17b.** Illustration of 16 and 77 unique REVIGO gene ontology biological processes associated with 159 and 336 GO terms extracted from 66 and 80 non-redundant genes that were identified by RAI<sub>i</sub>SD MuStat estimates of 20 standard deviations in the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively.

### **C8. Analysis for climate-specific groups**

**Fig. S18.** The proportions of significant sliding windows per chromosome with 9 standard deviations of RAI<sub>i</sub>SD MuStat (pSWC9) in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S19.** The proportions of significant sliding windows per chromosome with 15 standard deviations of RAI<sub>i</sub>SD MuStat (pSWC15) in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S20.** Comparative allele frequency distributions for all the SNPs detected in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008.

**Fig. S21.** Comparative allele frequency distributions for all the deleterious SNPs (dSNPs) detected in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008.



**Table S2a.** Summary of exome capture sequence reads for each sequenced sample of emmer wheat collected in 1980.

Exome Capture Sample	Lane	fastq R1	fastq R2	total reads in bam file	total mapped reads in bam file	Exome Capture Sample	Lane	fastq R1	fastq R2	total reads in bam file	total mapped reads in bam file
e1980											
1980-01-05-FT	2	908,486	908,486	1,719,187	1,697,515	1980-06-01-FT	2	2,260,778	2,260,778	4,264,419	4,213,866
1980-01-06-FT	2	1,862,460	1,862,460	3,496,499	3,457,180	1980-06-03-FT	2	2,073,104	2,073,104	3,929,228	3,880,540
1980-01-08-FT	2	2,608,902	2,608,902	4,922,073	4,864,410	1980-06-07-FT	2	1,073,246	1,073,246	1,697,879	1,676,720
1980-01-09-FT	2	2,421,518	2,421,518	4,511,255	4,465,133	1980-06-08-FT	2	2,012,931	2,012,931	3,762,846	3,719,439
1980-01-10-FT	3	2,645,437	2,645,437	5,031,853	4,968,071	1980-06-09-FT	2	2,539,541	2,539,541	4,748,489	4,702,120
1980-01-16-ISR	3	2,411,172	2,411,172	4,571,096	4,516,922	1980-06-10-FT	3	2,637,722	2,637,722	5,009,048	4,946,398
1980-01-20-ISR	3	2,363,967	2,363,967	4,501,147	4,441,207	1980-06-12-ISR	3	1,637,978	1,637,978	3,111,061	3,069,140
1980-02-02-FT	2	1,526,145	1,526,145	2,904,990	2,869,958	1980-07-01-FT	2	2,705,128	2,705,128	5,072,259	5,019,439
1980-02-03-FT	2	2,436,434	2,436,434	4,562,781	4,518,925	1980-07-02-FT	2	2,453,226	2,453,226	4,605,760	4,556,146
1980-02-04-FT	2	2,122,546	2,122,546	3,984,001	3,940,836	1980-07-03-FT	3	2,317,845	2,317,845	4,403,626	4,349,284
1980-02-05-FT	2	2,110,594	2,110,594	3,976,462	3,933,625	1980-07-06-FT	2	2,246,357	2,246,357	4,223,394	4,180,952
1980-02-06-FT	3	3,140,799	3,140,799	5,943,161	5,875,468	1980-07-09-FT	2	1,900,136	1,900,136	3,583,459	3,542,589
1980-02-13-ISR	3	1,746,968	1,746,968	3,313,821	3,272,611	1980-07-10-FT	2	2,167,060	2,167,060	4,095,623	4,041,248
1980-02-14-ISR	3	2,826,274	2,826,274	5,364,452	5,294,964	1980-07-14-ISR	3	2,624,901	2,624,901	5,000,418	4,936,419
1980-02-17-ISR	3	3,134,860	3,134,860	5,936,675	5,861,402	1980-07-19-ISR	3	2,391,802	2,391,802	4,534,238	4,505,261
1980-02-19-ISR	3	2,554,698	2,554,698	4,863,909	4,784,611	1980-08-04-FT	2	2,012,508	2,012,508	3,787,407	3,746,611
1980-03-01-ISR	2	2,097,008	2,097,008	3,946,377	3,917,571	1980-08-05-FT	2	2,214,731	2,214,731	4,167,849	4,120,031
1980-03-02-FT	2	2,254,766	2,254,766	4,223,741	4,200,147	1980-08-06-FT	2	1,447,466	1,447,466	2,732,259	2,699,175
1980-03-03-FT	2	2,155,154	2,155,154	4,051,650	4,025,754	1980-08-06-ISR	3	2,944,229	2,944,229	5,582,277	5,511,480
1980-03-04-ISR	2	2,401,450	2,401,450	4,493,849	4,465,778	1980-08-07-FT	2	2,249,073	2,249,073	4,021,793	3,974,058
1980-03-05-ISR	2	2,401,739	2,401,739	4,506,710	4,474,384	1980-08-08-FT	3	2,473,793	2,473,793	4,693,527	4,632,921
1980-03-07-ISR	3	2,539,234	2,539,234	4,793,911	4,765,634	1980-08-09-FT	3	2,609,106	2,609,106	4,949,782	4,894,762
1980-03-08-ISR	3	2,858,208	2,858,208	5,375,848	5,338,400	1980-08-12-ISR	3	2,941,190	2,941,190	5,580,675	5,512,632
1980-03-10-ISR	3	2,005,212	2,005,212	3,788,941	3,760,311	1980-08-15-ISR	3	2,786,675	2,786,675	5,295,837	5,225,909
1980-03-11-ISR	3	1,758,837	1,758,837	3,324,054	3,299,269	1980-08-17-ISR	2	1,728,897	1,728,897	3,264,845	3,220,533
1980-03-12-ISR	3	2,896,630	2,896,630	5,461,991	5,428,733	1980-09-02-FT	2	3,036,352	3,036,352	5,658,481	5,631,561
1980-04-01-ISR	2	2,040,963	2,040,963	3,339,584	3,317,230	1980-09-03-FT	2	2,709,785	2,709,785	5,060,088	5,034,222
1980-04-02-FT	2	2,563,533	2,563,533	4,807,497	4,761,267	1980-09-04-FT	2	2,856,279	2,856,279	5,324,271	5,286,805
1980-04-02-ISR	2	2,803,036	2,803,036	5,250,432	5,193,665	1980-09-05-FT	2	2,651,382	2,651,382	4,958,053	4,934,270
1980-04-07-ISR	2	2,344,283	2,344,283	4,385,377	4,344,755	1980-09-06-FT	2	2,153,196	2,153,196	4,031,165	4,008,437
1980-04-08-ISR	2	2,316,289	2,316,289	4,350,100	4,305,091	1980-09-06-ISR	3	2,530,805	2,530,805	4,783,865	4,740,772
1980-04-09-ISR	3	2,111,543	2,111,543	4,027,026	3,981,041	1980-09-08-ISR	3	1,887,040	1,887,040	3,583,463	3,542,775
1980-04-10-ISR	3	2,652,036	2,652,036	5,038,208	4,980,407	1980-09-13-ISR	3	1,603,549	1,603,549	3,029,420	3,009,652
1980-04-12-ISR	3	2,458,872	2,458,872	4,691,641	4,632,547	1980-09-17-ISR	3	2,618,809	2,618,809	4,944,110	4,915,477
1980-04-15-ISR	3	3,015,984	3,015,984	5,724,038	5,650,623	1980-09-20-ISR	3	2,404,876	2,404,876	4,542,487	4,514,493
1980-04-18-ISR	3	2,647,267	2,647,267	5,026,029	4,961,579	1980-10-02-FT	2	2,099,752	2,099,752	3,937,571	3,915,082
1980-05-02-FT	2	1,955,502	1,955,502	3,654,650	3,632,029	1980-10-03-FT	2	2,218,642	2,218,642	4,166,644	4,136,390
1980-05-03-FT	2	2,063,834	2,063,834	3,891,327	3,847,599	1980-10-04-FT	2	2,279,788	2,279,788	4,281,714	4,243,401
1980-05-05-FT	2	1,992,739	1,992,739	3,762,149	3,719,276	1980-10-05-FT	2	2,173,645	2,173,645	4,073,833	4,046,713
1980-05-06-FT	2	2,338,139	2,338,139	4,401,346	4,349,475	1980-10-06-FT	2	2,055,849	2,055,849	3,855,817	3,831,026
1980-05-07-ISR	3	2,860,301	2,860,301	5,411,249	5,342,013	1980-10-06-ISR	3	2,401,842	2,401,842	4,555,025	4,525,171
1980-05-08-FT	2	1,795,025	1,795,025	3,379,967	3,339,707	1980-10-09-ISR	3	2,703,565	2,703,565	5,092,200	5,059,658
1980-05-09-FT	3	2,814,486	2,814,486	5,331,253	5,260,680	1980-10-14-ISR	3	2,614,996	2,614,996	4,947,983	4,915,181
1980-05-10-FT	3	1,618,099	1,618,099	3,068,250	3,026,994	1980-10-18-ISR	3	3,121,190	3,121,190	5,882,549	5,842,852
1980-05-18-ISR	3	2,161,052	2,161,052	4,109,625	4,060,639	1980-10-20-ISR	3	2,962,252	2,962,252	5,586,170	5,544,396
1980-05-19-ISR	3	2,904,623	2,904,623	5,519,835	5,448,331						

**Table S2b.** Summary of exome capture sequence reads for each sequenced sample of emmer wheat collected in 2008.

Exome Capture Sample	Lane	fastq R1	fastq R2	total reads in bam file	total mapped reads in bam file	Exome Capture Sample	Lane	fastq R1	fastq R2	total reads in bam file	total mapped reads in bam file
e2008											
2008-01-13-FT	2	1,514,240	1,514,240	2,873,251	2,841,032	2008-06-08-ISR	3	2,499,922	2,499,922	4,727,259	4,676,034
2008-01-16-FT	2	2,791,751	2,791,751	5,232,189	5,197,581	2008-06-11-ISR	3	2,892,778	2,892,778	5,491,171	5,416,031
2008-01-16-ISR	2	1,042,580	1,042,580	1,971,820	1,948,830	2008-06-12-FT	2	2,850,621	2,850,621	5,333,962	5,275,851
2008-01-17-FT	3	2,403,320	2,403,320	4,543,270	4,512,913	2008-06-13-FT	2	2,249,158	2,249,158	4,219,566	4,172,059
2008-01-18-FT	3	2,450,853	2,450,853	4,625,670	4,596,425	2008-06-17-ISR	2	2,195,682	2,195,682	4,132,197	4,086,448
2008-01-19-FT	3	2,343,650	2,343,650	4,435,278	4,401,476	2008-06-19-ISR	2	1,081,816	1,081,816	2,051,236	2,026,183
2008-02-02-ISR	3	1,902,853	1,902,853	3,614,445	3,565,795	2008-06-20-ISR	2	901,780	901,780	1,430,842	1,413,022
2008-02-11-FT	2	2,462,223	2,462,223	4,638,562	4,591,858	2008-07-02-ISR	3	1,942,925	1,942,925	3,690,101	3,637,925
2008-02-12-ISR	3	2,330,157	2,330,157	4,423,428	4,363,642	2008-07-03-ISR	3	1,769,768	1,769,768	3,360,744	3,317,124
2008-02-14-FT	2	1,961,255	1,961,255	3,680,384	3,645,387	2008-07-05-ISR	3	2,807,507	2,807,507	5,325,788	5,265,459
2008-02-14-ISR	3	3,671,067	3,671,067	6,912,044	6,840,530	2008-07-06-ISR	3	2,528,083	2,528,083	4,805,937	4,741,698
2008-02-16-FT	2	2,232,451	2,232,451	4,208,445	4,161,802	2008-07-10-ISR	3	2,586,606	2,586,606	4,913,823	4,855,688
2008-02-17-FT	2	1,975,238	1,975,238	3,732,414	3,690,650	2008-07-11-FT	2	2,529,257	2,529,257	4,752,187	4,691,583
2008-02-17-ISR	3	2,868,008	2,868,008	5,420,786	5,354,410	2008-07-14-ISR	2	2,621,847	2,621,847	4,890,995	4,846,455
2008-02-19-ISR	3	2,442,918	2,442,918	4,651,819	4,592,579	2008-07-16-ISR	2	405,331	405,331	773,554	567,515
2008-02-20-ISR	2	2,604,682	2,604,682	4,876,411	4,826,562	2008-07-18-ISR	2	2,432,473	2,432,473	4,578,734	4,527,937
2008-03-01-ISR	3	2,397,869	2,397,869	4,553,803	4,504,006	2008-07-19-ISR	2	2,265,915	2,265,915	4,265,213	4,220,678
2008-03-02-ISR	3	2,843,717	2,843,717	5,390,239	5,320,919	2008-08-02-ISR	3	2,680,454	2,680,454	5,053,639	5,019,494
2008-03-03-ISR	3	2,656,071	2,656,071	5,041,901	4,976,486	2008-08-05-ISR	3	2,746,393	2,746,393	5,185,566	5,146,280
2008-03-07-ISR	3	2,707,443	2,707,443	5,148,649	5,080,258	2008-08-09-ISR	3	398,122	398,122	769,276	522,433
2008-03-08-ISR	3	1,995,112	1,995,112	3,792,837	3,746,785	2008-08-11-ISR	3	2,653,220	2,653,220	5,015,933	4,975,451
2008-03-09-ISR	2	2,017,126	2,017,126	3,811,775	3,760,496	2008-08-12-ISR	3	2,795,039	2,795,039	5,273,249	5,234,320
2008-03-10-ISR	2	2,046,082	2,046,082	3,865,576	3,818,569	2008-08-13-ISR	2	2,157,659	2,157,659	4,039,847	4,009,471
2008-03-12-FT	2	1,365,403	1,365,403	2,578,478	2,547,997	2008-08-14-ISR	2	2,339,729	2,339,729	4,385,701	4,355,842
2008-03-15-ISR	2	1,807,890	1,807,890	3,411,259	3,369,839	2008-08-16-ISR	2	2,047,110	2,047,110	3,843,965	3,819,007
2008-03-17-ISR	2	2,221,387	2,221,387	4,188,703	4,139,716	2008-08-18-ISR	2	1,054,834	1,054,834	1,988,444	1,975,540
2008-04-11-FT	2	2,667,698	2,667,698	4,973,779	4,946,952	2008-08-20-ISR	2	2,226,612	2,226,612	3,840,990	3,816,041
2008-04-12-FT	2	2,566,077	2,566,077	4,787,685	4,764,411	2008-09-02-ISR	3	2,640,384	2,640,384	4,974,219	4,941,424
2008-04-13-FT	2	2,097,783	2,097,783	3,750,966	3,733,330	2008-09-07-ISR	3	2,206,871	2,206,871	4,165,916	4,133,344
2008-04-16-FT	2	2,453,140	2,453,140	4,597,509	4,572,997	2008-09-08-ISR	3	2,356,891	2,356,891	4,449,776	4,414,848
2008-04-17-FT	3	2,344,394	2,344,394	4,432,922	4,405,791	2008-09-10-ISR	3	2,656,697	2,656,697	5,007,526	4,975,622
2008-04-18-FT	3	2,158,831	2,158,831	4,088,393	4,059,327	2008-09-11-ISR	3	2,842,044	2,842,044	5,355,099	5,320,640
2008-04-19-FT	3	1,570,278	1,570,278	2,962,768	2,944,564	2008-09-12-FT	2	2,305,409	2,305,409	4,319,466	4,298,227
2008-04-20-ISR	2	2,282,425	2,282,425	3,602,819	3,577,937	2008-09-13-FT	2	2,301,850	2,301,850	4,310,350	4,287,308
2008-04-20-FT	3	1,912,223	1,912,223	4,270,288	4,228,108	2008-09-15-ISR	2	2,473,575	2,473,575	4,617,521	4,594,829
2008-05-04-ISR	3	2,560,586	2,560,586	4,861,083	4,801,411	2008-09-18-ISR	2	2,156,641	2,156,641	4,040,113	4,015,433
2008-05-09-ISR	3	2,064,566	2,064,566	3,901,521	3,851,334	2008-09-20-ISR	2	1,872,898	1,872,898	3,530,521	3,512,760
2008-05-12-FT	2	2,641,147	2,641,147	4,983,030	4,915,327	2008-10-01-ISR	3	2,809,513	2,809,513	5,321,095	5,258,519
2008-05-12-ISR	3	2,521,367	2,521,367	4,796,941	4,738,308	2008-10-03-ISR	3	3,102,028	3,102,028	5,835,706	5,773,823
2008-05-13-FT	2	1,999,676	1,999,676	3,768,011	3,723,692	2008-10-05-ISR	3	2,725,569	2,725,569	5,165,056	5,105,879
2008-05-14-ISR	2	2,512,241	2,512,241	4,720,357	4,671,395	2008-10-06-ISR	3	3,234,251	3,234,251	6,104,337	6,033,797
2008-05-14-FT	3	2,776,459	2,776,459	5,247,100	5,188,848	2008-10-13-FT	2	2,537,617	2,537,617	4,749,147	4,714,242
2008-05-15-FT	3	2,267,521	2,267,521	4,306,710	4,255,139	2008-10-14-FT	2	2,214,242	2,214,242	4,145,059	4,114,483
2008-05-17-ISR	2	2,253,348	2,253,348	4,249,465	4,200,822	2008-10-15-FT	2	2,229,403	2,229,403	4,178,544	4,150,665
2008-05-19-ISR	2	2,004,043	2,004,043	3,779,473	3,730,584	2008-10-16-FT	3	2,867,538	2,867,538	5,397,714	5,362,527
2008-06-03-ISR	3	2,638,478	2,638,478	5,013,891	4,952,762	2008-10-17-ISR	2	2,289,962	2,289,962	4,323,754	4,276,206
2008-06-04-ISR	3	2,412,084	2,412,084	4,576,369	4,518,617	2008-10-19-ISR	2	2,288,965	2,288,965	4,323,492	4,266,244
2008-06-05-ISR	3	2,821,922	2,821,922	5,334,611	5,271,908						

**Table S3.** List of plant species used to generate GERP++ RS scores for identification of deleterious SNPs in wild emmer wheat samples.

genome sequence FASTA file	Common Name	Source	Wild Emmer	
			GERP 12	Ancestor
Arabidopsis_thaliana.TAIR10.dna_rm.toplevel.fa	thale	Ensembl	●	
Brachypodium_distachyon.v1.0.dna_rm.toplevel.fa	brome	Ensembl	●	
Musa_acuminata.MA1.dna_rm.toplevel.fa	banana	Ensembl	●	
Oryza_indica-ASMv1.dna_rm.toplevel.fa	indica	Ensembl	●	
Phaseolus_vulgaris.PhaVulg1_0.dna_rm.toplevel.fa	bean	Ensembl	●	
Sorghum_bicolor.Sorghum_bicolor_NCBIv3.dna_rm.toplevel.fa	sorghum	Ensembl	●	
Zea_mays.AGPv4.dna_rm.toplevel.fa.gz	maize	Ensembl	●	
Beta_vulgaris.RefBeet-1.2.2.dna_rm.toplevel.fa	beet	Ensembl	●	
Trifolium_pratense.Trpr.dna_rm.toplevel.fa	clover	Ensembl	●	
Vitis_vinifera.IGGP_12x.dna_rm.toplevel.fa	grape	Ensembl	●	
Hordeum_vulgare.Hv_IBSC_PGSB_v2.dna.toplevel.fa	barley	Ensembl	●	
Triticum_urartu.ASM34745v1.dna_rm.toplevel.fa	urartu	Ensembl	●	●

**Table S4.** SNP discovery and annotation in the samples of wild emmer wheat collected in 1980 and in 2008.

Variant	1980	2008
<b>SNP calling using ANGSD</b>		
Total SNPs	6,499,444	6,482,132
<b>SNP annotation with VEP* (all consequences)</b>		
Missence_variant (MV)	986,903	956,002
Proportion of MV	0.152	0.147
Synonymous_variant (SV)	899,855	874,833
Porportion of SV	0.138	0.135
<b>SNP annotation with VEP* (most severe consequences)</b>		
Splice_acceptor_variant	2,116	2,039
Splice_donor_variant	1,950	1,880
Stop_gained	8,031	7,787
Stop_lost	2,059	2,044
Start_lost	2,149	2,137
Missense_variant	290,514	284,028
Splice_region_variant	27,017	26,098
Synonymous_variant	223,910	218,929
Stop_retained_variant	498	498
Coding_sequence_variant	0	0
5_prime_UTR_variant	134,265	131,597
3_prime_UTR_variant	196,857	191,036
Non_coding_transcript_exon_variant	2,084	2,035
Intron_variant	555,211	551,604
Upstream_gene_variant	751,814	757,564
Downstream_gene_variant	485,980	491,106
Intergenic_variant	3,651,358	3,647,748
<b>SIFT analysis with CTD**</b>		
SIFT-deleterious SNPs (SDS)	43,077	41,233
Proportion of SDS	0.006628	0.006361
Deleterious_low_confidence SNPs	18,149	17,707
Tolerated SNPs	371155	363182
<b>Deleterious SNPs filtered by SIFT by SIFT+RS</b>		
SDS+12RS-filtered SNPs (12RSD)	20,827	19,658
Proportion of 12RSD	0.003204	0.003033
12RSD after genotyping with ancestor (12RSDa)	19,672	18,627
Proportion of 12RSDa	0.003027	0.002874
Fixed 12RSDa	105	104
Proportion of fixed 12RSDa	0.000016	0.000016
* VEP analysis did not include the chrUn sequences		
** SIFT-filtered with canonical transcripts		



**Table S5.** Summary statistics and test significance for the mean Watterson’s theta estimates per chromosome (aWc) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. There were 24 Kruskal–Wallis one-way ANOVA tests with respect to population (2), climate group (4) and year (18). A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively. For example, a significant decline in aWc from 1980 to 2008 was found for population 10, but not for population 2. Significant differences in aWc were observed among the populations or groups in either 1980 or 2008 collection. Overall, aWc showed a significant reduction from 1980 to 2008.

	Sample size	Mean(aWc)	STD(aWc)	Sample size	Mean(aWc)	STD(aWc)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.00234	0.00023	6	0.00194	0.00016	-0.00040
2	9	0.00178	0.00022	10	0.00174	0.00021	-0.00003
3	10	0.00230	0.00019	9	0.00170	0.00018	-0.00061
4	10	0.00113	0.00025	10	0.00173	0.00016	0.00060
5	10	0.00077	0.00015	10	0.00098	0.00023	<b>0.00021</b>
6	10	0.00101	0.00026	10	0.00228	0.00016	0.00126
7	10	0.00223	0.00019	10	0.00111	0.00013	-0.00112
8	7	0.00206	0.00021	10	0.00171	0.00015	-0.00035
9	8	0.00271	0.00026	9	0.00222	0.00023	-0.00049
10	10	0.00277	0.00023	9	0.00041	0.00010	-0.00236
mean		0.00191	0.00022		0.00158	0.00017	-0.00033
<i>Group</i>							
rain1	45	0.00268	0.00021	49	0.00296	0.00024	<b>0.00027</b>
rain2	29	0.00306	0.00021	29	0.00259	0.00018	-0.00047
rain3	17	0.00293	0.00024	15	0.00193	0.00018	-0.00101
temp1	26	0.00309	0.00027	25	0.00229	0.00015	-0.00080
temp2	45	0.00306	0.00024	48	0.00305	0.00025	-0.00001
temp3	20	0.00101	0.00021	20	0.00219	0.00016	0.00117
<i>All</i>	<b>91</b>	<b>0.00310</b>	<b>0.00021</b>	<b>93</b>	<b>0.00292</b>	<b>0.00021</b>	<b>-0.00017</b>

**Table S6.** Summary statistics and test significance for the mean nucleotide diversity Pi estimates per chromosome (aPc) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	Mean(aPc)	STD(aPc)	Sample size	Mean(aPc)	STD(aPc)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.00298	0.00030	6	0.00229	0.00020	-0.00068
2	9	0.00237	0.00030	10	0.00234	0.00027	-0.00003
3	10	0.00247	0.00021	9	0.00166	0.00024	-0.00081
4	10	0.00121	0.00031	10	0.00204	0.00020	0.00084
5	10	0.00100	0.00016	10	0.00119	0.00030	<b>0.00019</b>
6	10	0.00127	0.00035	10	0.00342	0.00023	0.00215
7	10	0.00254	0.00022	10	0.00152	0.00017	-0.00102
8	7	0.00257	0.00027	10	0.00261	0.00023	0.00004
9	8	0.00312	0.00033	9	0.00290	0.00031	<b>-0.00022</b>
10	10	0.00333	0.00031	9	0.00060	0.00014	-0.00273
Mean		0.00229	0.00027		0.00206	0.00023	-0.00023
<i>Group</i>							
rain1	45	0.00400	0.00026	49	0.00464	0.00037	0.00064
rain2	29	0.00420	0.00029	29	0.00416	0.00028	-0.00003
rain3	17	0.00353	0.00033	15	0.00200	0.00025	-0.00154
temp1	26	0.00393	0.00038	25	0.00347	0.00019	<b>-0.00046</b>
temp2	45	0.00453	0.00032	48	0.00453	0.00040	0.00000
temp3	20	0.00130	0.00025	20	0.00318	0.00021	0.00188
<i>All</i>	91	0.00476	0.00027	93	0.00476	0.00029	0.00000

**Table S7.** Summary statistics and test significance for mean individual inbreeding coefficient ( $F_{is}$ ) and population differentiation over years ( $F_{st}$ ) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	Mean( $F_{is}$ )	STD ( $F_{is}$ )	Sample size	Mean( $F_{is}$ )	STD ( $F_{is}$ )	Difference	$F_{st}$	STD( $F_{st}$ )
	1980			2008				Over years	
<i>Population</i>									
1	7	0.360	0.116	6	0.613	0.117	0.254	0.335	0.017
2	9	0.157	0.185	10	0.168	0.240	0.010	0.345	0.020
3	10	0.184	0.331	9	0.547	0.156	0.363	0.526	0.022
4	10	0.422	0.152	10	0.134	0.283	-0.287	0.684	0.033
5	10	0.357	0.139	10	0.328	0.252	-0.029	0.265	0.037
6	10	0.420	0.143	10	0.459	0.357	0.038	0.388	0.024
7	10	0.152	0.287	10	0.046	0.144	-0.106	0.320	0.049
8	7	0.288	0.170	10	0.194	0.136	-0.093	0.143	0.019
9	8	0.399	0.264	9	0.325	0.160	-0.074	0.167	0.024
10	10	0.372	0.168	9	0.052	0.082	-0.320	0.591	0.025
mean		0.311	0.196		0.287	0.193	-0.024	0.376	0.027
<i>Group</i>									
rain1	45	0.684	0.140	49	0.621	0.169	-0.063	0.064	0.002
rain2	29	0.650	0.165	29	0.626	0.156	-0.024	0.092	0.005
rain3	17	0.429	0.226	15	0.642	0.136	0.213	0.390	0.020
temp1	26	0.520	0.107	25	0.670	0.117	0.150	0.238	0.008
temp2	45	0.642	0.159	48	0.586	0.164	-0.056	0.051	0.002
temp3	20	0.508	0.094	20	0.680	0.100	0.172	0.177	0.011
<i>All</i>	91	0.688	0.140	93	0.687	0.133	-0.001	0.021	0.001

**Table S8.** Summary statistics and test significance for the proportions of significant sliding windows per chromosome with 9 standard deviations of RAiSD MuStat (pSWC9) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	Mean (pSWC9)	SD (pSWC9)	Sample size	Mean (pSWC9)	SD (pSWC9)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.001287	0.000694	6	0.001407	0.000488	0.00012
2	9	0.001584	0.000566	10	0.001599	0.000590	0.00001
3	10	0.000717	0.000364	9	0.001447	0.000581	0.00073
4	10	0.001905	0.001080	10	0.001150	0.000509	-0.00076
5	10	0.002095	0.000905	10	0.001876	0.000847	-0.00022
6	10	0.002105	0.000873	10	0.001153	0.000383	-0.00095
7	10	0.000603	0.000284	10	0.000585	0.000441	-0.00002
8	7	0.001719	0.000586	10	0.001697	0.000403	-0.00002
9	8	0.000605	0.000333	9	0.002078	0.000653	0.00147
10	10	0.001454	0.000556	9	0.001123	0.001032	-0.00033
Mean		0.001407	0.000624		0.001411	0.000593	0.000004
<i>Group</i>							
rain1	45	0.001942	0.000319	49	0.001518	0.000535	-0.00042
rain2	29	0.001611	0.000302	29	0.001806	0.000582	0.00020
rain3	17	0.001112	0.000387	15	0.001670	0.000450	0.00056
temp1	26	0.001876	0.000424	25	0.001548	0.000321	-0.00033
temp2	45	0.001740	0.000418	48	0.001747	0.000354	0.00001
temp3	20	0.002253	0.000514	20	0.001675	0.000456	-0.00058
<i>All</i>	91	0.001733	0.000454	93	0.002068	0.000331	0.00033

**Table S9.** Summary statistics and test significance for the proportions of significant sliding windows per chromosome with 15 standard deviations of RAiSD MuStat (pSWC15) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	Mean (pSWC15)	SD (pSWC15)	Sample size	Mean (pSWC15)	SD (pSWC15)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.000175	0.000330	6	0.000098	0.000120	-0.00008
2	9	0.000088	0.000155	10	0.000223	0.000265	0.00013
3	10	0.000054	0.000127	9	0.000148	0.000217	0.00009
4	10	0.000363	0.000452	10	0.000110	0.000166	-0.00025
5	10	0.000525	0.000469	10	0.000221	0.000252	-0.00030
6	10	0.000269	0.000357	10	0.000196	0.000213	-0.00007
7	10	0.000022	0.000036	10	0.000002	0.000007	-0.00002
8	7	0.000099	0.000198	10	0.000072	0.000118	-0.00003
9	8	0.000050	0.000134	9	0.000149	0.000178	0.00010
10	10	0.000094	0.000157	9	0.000011	0.000040	-0.00008
Mean		0.000174	0.000242		0.000123	0.000158	-0.000051
<i>Group</i>							
rain1	45	0.000374	0.000172	49	0.000428	0.000180	0.00005
rain2	29	0.000235	0.000077	29	0.000452	0.000217	0.00022
rain3	17	0.000033	0.000067	15	0.000183	0.000237	0.00015
temp1	26	0.000317	0.000217	25	0.000352	0.000205	0.00003
temp2	45	0.000318	0.000174	48	0.000387	0.000141	0.00007
temp3	20	0.000478	0.000370	20	0.000309	0.000251	-0.00017
<i>All</i>	91	0.000349	0.000111	93	0.000445	0.000160	0.00010

**Table S10.** Summary statistics and test significance for the proportions of sliding windows per chromosome with positive Tajima’s D statistics greater than 3 standard deviations (PSW>3D) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	mean(PSW>3D)	STD (PSW>3D)	Sample size	mean(PSW>3D)	STD (PSW>3D)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.00126	0.00173	6	0.00161	0.00154	0.00035
2	9	0.00164	0.00296	10	0.00319	0.00398	0.00155
3	10	0.01463	0.00377	9	0.01448	0.00639	-0.00015
4	10	0.01255	0.00684	10	0.02015	0.00561	0.00760
5	10	0.01685	0.00789	10	0.01505	0.00739	-0.00180
6	10	0.01057	0.00548	10	0.00000	0.00000	-0.01057
7	10	0.00318	0.00312	10	0.01774	0.00937	0.01456
8	7	0.00541	0.00466	10	0.00007	0.00024	-0.00535
9	8	0.00387	0.00301	9	0.00141	0.00238	-0.00246
10	10	0.01045	0.00769	9	0.02832	0.00865	0.01787
mean		0.00804	0.00472		0.01020	0.00455	0.00216
<i>Group</i>							
rain1	45	0.00131	0.00131	49	0.00076	0.00068	-0.00055
rain2	29	0.00110	0.00107	29	0.00001	0.00004	-0.00109
rain3	17	0.00298	0.00135	15	0.01341	0.00378	0.01044
temp1	26	0.00391	0.00163	25	0.00012	0.00024	-0.00378
temp2	45	0.00118	0.00095	48	0.00227	0.00080	0.00109
temp3	20	0.01121	0.00668	20	0.00018	0.00026	-0.01103
<i>All</i>	91	0.00156	0.00146	93	0.00106	0.00114	-0.00050

**Table S11.** Summary statistics and test significance for the proportions of sliding windows per chromosome with negative Tajima’s D statistics (PSW<0) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	mean(PSW<0)	STD (PSW<0)	Sample size	mean(PSW<0)	STD (PSW<0)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.43041	0.07616	6	0.45514	0.06527	0.02474
2	9	0.58345	0.07420	10	0.50643	0.07423	-0.07702
3	10	0.73795	0.03672	9	0.79335	0.06513	0.05540
4	10	0.76444	0.08883	10	0.71823	0.03639	-0.04621
5	10	0.72505	0.06674	10	0.72636	0.07341	0.00130
6	10	0.68858	0.08530	10	0.27020	0.04461	-0.41838
7	10	0.67734	0.05597	10	0.77638	0.03200	0.09904
8	7	0.55613	0.06765	10	0.31034	0.08484	-0.24580
9	8	0.54121	0.07683	9	0.46696	0.07956	-0.07425
10	10	0.46227	0.07812	9	0.80525	0.06012	0.34298
mean		0.61668	0.07065		0.58286	0.06155	-0.03382
<i>Group</i>							
rain1	45	0.19287	0.03239	49	0.14281	0.03063	-0.05006
rain2	29	0.23304	0.03456	29	0.16307	0.03507	-0.06997
rain3	17	0.46915	0.05982	15	0.70135	0.05953	0.23221
temp1	26	0.36377	0.06178	25	0.23544	0.04029	-0.12833
temp2	45	0.16966	0.03092	48	0.16051	0.03622	-0.00915
temp3	20	0.66202	0.06959	20	0.22627	0.03795	-0.43575
<i>All</i>	<b>91</b>	<b>0.15665</b>	<b>0.02600</b>	<b>93</b>	<b>0.13485</b>	<b>0.02645</b>	<b>-0.02180</b>

**Table S12.** Summary counts of total SNPs, total deleterious SNPs and proportional deleterious SNPs (PDS) identified in wild emmer wheat collected in 1980 and in 2008, with respect to population, climate group and year. The difference in PDS between two sampling years was also shown.

	Sample size	Total SNPs	Total Del SNPs	Prop of Del SNPs	Sample size	Total SNPs	Total Del SNPs	Prop of Del SNPs	comparison
	1980				2008				
<i>Population</i>									
1	7	707,081	2,726	0.000551	6	1,344,410	4,950	0.000614	0.0000629
2	9	1,059,248	4,278	0.000449	10	502,038	2,331	0.000464	0.0000156
3	10	643,857	2,613	0.000406	9	1,397,441	4,703	0.000374	-0.0000319
4	10	1,409,202	4,709	0.000334	10	1,496,123	5,004	0.000334	0.0000003
5	10	1,475,135	6,109	0.000414	10	566,066	1,927	0.000340	-0.0000737
6	10	655,527	2,406	0.000367	10	668,598	2,651	0.000397	0.0000295
7	10	1,440,611	4,992	0.000347	10	609,899	2,750	0.000451	0.0001044
8	7	1,382,675	4,722	0.000488	10	1,436,377	4,420	0.000308	-0.0001802
9	8	965,873	4,278	0.000554	9	1,438,379	5,275	0.000407	-0.0001462
10	10	1,087,735	4,872	0.000448	9	1,390,281	5,434	0.000434	-0.0000136
mean		1,082,694	4,171	0.000436		1,084,961	3,945	0.000412	-0.0000233
<i>Group</i>									
rain1	45	3,112,876	11,612	0.000083	49	4,162,447	13,363	0.000066	-0.0000174
rain2	29	2,655,963	10,107	0.000131	29	2,628,828	8,997	0.000118	-0.0000132
rain3	17	2,099,453	7,624	0.000214	15	941,063	4,213	0.000298	0.0000848
temp1	26	3,002,809	10,690	0.000137	25	1,971,055	7,163	0.000145	0.0000084
temp2	45	3,662,142	12,983	0.000079	48	4,189,743	13,480	0.000067	-0.0000118
temp3	20	1,130,474	3,768	0.000167	20	1,684,487	6,369	0.000189	0.0000224
<i>All</i>	91	6,499,444	19,672	0.000033	93	6,482,132	18,627	0.000031	-0.0000024



**Table S13.** Summary statistics and test significance for the mean counts of deleterious SNPs per chromosome (delSNPc) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	Mean(delSNPc)	STD(DeISNPc)	Sample size	Mean(delSNPc)	STD(DeISNPc)	Difference
	1980			2008			
<i>Population</i>							
1	7	305.6	77.5	6	166.5	40.9	-139.1
2	9	336.4	67.4	10	357.4	66.9	21.1
3	10	356.6	66.4	9	196.4	62.8	-160.1
4	10	186.6	47.4	10	335.9	64.8	149.3
5	10	171.9	38.6	10	189.4	54.8	17.5
6	10	194.7	49.2	10	353.6	79.7	158.9
7	10	337.3	77.2	10	315.7	70.5	-21.6
8	7	305.6	60.8	10	376.8	86.8	71.2
9	8	348.0	84.8	9	388.1	84.9	40.1
10	10	436.4	102.7	9	137.6	30.3	-298.7
mean		297.9	67.2		281.8	64.2	-16.1
<i>Group</i>							
rain1	45	829.4	176.0	49	954.5	213.2	125.1
rain2	29	721.9	153.4	29	642.6	116.6	-79.3
rain3	17	544.6	116.3	15	300.9	85.6	-243.6
temp1	26	763.6	166.6	25	511.6	94.6	-251.9
temp2	45	927.4	188.7	48	962.9	215.6	35.5
temp3	20	269.1	68.1	20	454.9	105.3	185.8
<i>All</i>	91	1405.1	301.1	93	1330.5	286.4	-74.6

**Table S14.** Summary statistics and test significance for the estimates of alpha-dfe by polyDFE (in 30 bootstrapped samples) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests were performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively. NA stands for non-achievable due to a large sample size for polyDFE.

	Sample size	alpha-dfe	STD (alpha-dfe)	Sample size	alpha-dfe	STD (alpha-dfe)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.412	0.014	6	0.815	0.024	0.403
2	9	0.503	0.013	10	0.776	0.036	0.273
3	10	0.873	0.012	9	0.866	0.010	-0.006
4	10	0.811	0.016	10	0.834	0.016	0.023
5	10	0.427	0.020	10	0.384	0.012	-0.042
6	10	0.412	0.011	10	0.887	0.019	0.475
7	10	0.828	0.038	10	0.855	0.019	0.027
8	7	0.774	0.068	10	0.568	0.016	-0.205
9	8	0.389	0.014	9	0.883	0.008	0.494
10	10	0.460	0.013	9	0.576	0.014	0.117
mean		0.589	0.022		0.745	0.017	0.156
<i>Group</i>							
rain1	45	0.658	0.008	49	0.547	0.021	-0.111
rain2	29	0.542	0.050	29	0.639	0.003	0.096
rain3	17	0.454	0.010	15	0.758	0.023	0.304
temp1	26	0.416	0.008	25	0.655	0.018	0.239
temp2	45	0.551	0.017	48	0.913	0.165	0.362
temp3	20	0.798	0.023	20	0.647	0.006	-0.150
<i>All</i>	91	NA		93	NA		NA

**Table S15.** Summary statistics and test significance for individual heterozygous load estimates per deleterious locus (Het load) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	Het load	STD (Het load)	Sample size	Het load	STD (Het load)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.0980	0.0161	6	0.1038	0.0169	0.0058
2	9	0.1134	0.0123	10	0.1132	0.0126	-0.0002
3	10	0.1118	0.0160	9	0.0936	0.0151	<b>-0.0182</b>
4	10	0.1053	0.0137	10	0.1138	0.0186	0.0084
5	10	0.1257	0.0091	10	0.1147	0.0107	<b>-0.0109</b>
6	10	0.1162	0.0118	10	0.1164	0.0092	0.0002
7	10	0.1222	0.0179	10	0.1221	0.0103	-0.0001
8	7	0.0949	0.0103	10	0.1202	0.0155	<b>0.0253</b>
9	8	0.0961	0.0071	9	0.1028	0.0044	<b>0.0067</b>
10	10	0.1014	0.0115	9	0.1378	0.0081	<b>0.0365</b>
mean		<b>0.1085</b>	<b>0.0126</b>		<b>0.1138</b>	<b>0.0121</b>	<b>0.0054</b>
<i>Group</i>							
rain1	45	0.0788	0.0184	49	0.0839	0.0139	0.0050
rain2	29	0.0852	0.0156	29	0.1050	0.0137	<b>0.0198</b>
rain3	17	0.1023	0.0091	15	0.0908	0.0124	<b>-0.0115</b>
temp1	26	0.0866	0.0074	25	0.1127	0.0236	<b>0.0261</b>
temp2	45	0.0774	0.0135	48	0.0829	0.0117	<b>0.0054</b>
temp3	20	0.1095	0.0067	20	0.0971	0.0320	<b>-0.0125</b>
<i>All</i>	91	0.0580	0.0115	93	0.0632	0.0135	<b>0.0052</b>

**Table S16.** Summary statistics and test significance for individual homozygous load estimates per deleterious locus (Hom load) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

	Sample size	Hom load	STD (Hom load)	Sample size	Hom load	STD (Hom load)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.1979	0.0274	6	0.1559	0.0180	-0.0421
2	9	0.2388	0.0234	10	0.2467	0.0214	0.0079
3	10	0.2462	0.0560	9	0.1206	0.0154	<b>-0.1257</b>
4	10	0.1601	0.0093	10	0.2829	0.0444	0.1228
5	10	0.1869	0.0101	10	0.1728	0.0170	-0.0142
6	10	0.1516	0.0177	10	0.1727	0.0702	0.0211
7	10	0.2432	0.0498	10	0.3223	0.0201	0.0790
8	7	0.2341	0.0211	10	0.2263	0.0142	-0.0078
9	8	0.1861	0.0490	9	0.2010	0.0123	<b>0.0149</b>
10	10	0.1541	0.0146	9	0.2759	0.0091	0.1217
mean		<b>0.1999</b>	<b>0.0278</b>		<b>0.2177</b>	<b>0.0242</b>	<b>0.0178</b>
<i>Group</i>							
rain1	45	0.0439	0.0149	49	0.0552	0.0170	0.0113
rain2	29	0.0613	0.0207	29	0.0827	0.0282	0.0214
rain3	17	0.1277	0.0291	15	0.0771	0.0101	-0.0506
temp1	26	0.0850	0.0087	25	0.0791	0.0288	-0.0059
temp2	45	0.0507	0.0166	48	0.0617	0.0164	0.0110
temp3	20	0.1127	0.0090	20	0.0804	0.0299	<b>-0.0322</b>
<i>All</i>	<b>91</b>	<b>0.0289</b>	<b>0.0091</b>	<b>93</b>	<b>0.0310</b>	<b>0.0105</b>	<b>0.0021</b>

**Table S17.** Summary statistics and test significance for individual total load estimates per deleterious locus (TA load) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

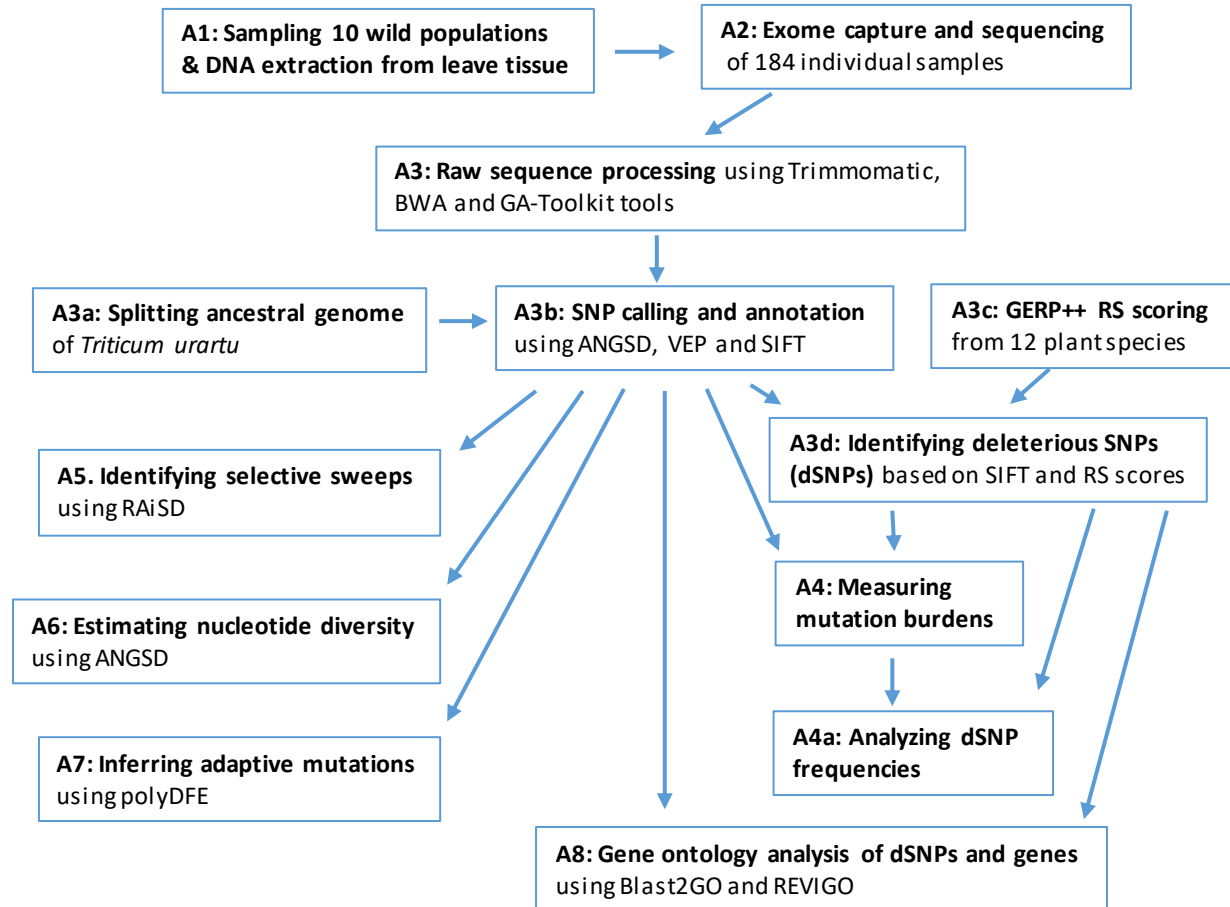
	Sample size	TA load	STD (TA load)	Sample size	TA load	STD (TA load)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.5083	0.0408	6	0.5545	0.3745	0.0463
2	9	0.5693	0.1160	10	0.5904	0.0613	0.0211
3	10	0.6060	0.1058	9	0.4242	0.2606	<b>-0.1818</b>
4	10	0.4377	0.0336	10	0.6533	0.1651	<b>0.2156</b>
5	10	0.4931	0.0201	10	0.5156	0.1741	0.0225
6	10	0.4198	0.0405	10	0.4152	0.1728	-0.0046
7	10	0.6244	0.0661	10	0.7587	0.0581	<b>0.1343</b>
8	7	0.5725	0.0505	10	0.5697	0.0315	-0.0028
9	8	0.4635	0.0950	9	0.4811	0.0714	0.0177
10	10	0.3921	0.0501	9	0.6754	0.0376	<b>0.2833</b>
mean		<b>0.5087</b>	<b>0.0618</b>		<b>0.5638</b>	<b>0.1407</b>	<b>0.0552</b>
<i>Group</i>							
rain1	45	0.1660	0.0454	49	0.1943	0.0449	0.0283
rain2	29	0.2084	0.0525	29	0.2665	0.0685	0.0581
rain3	17	0.3605	0.0557	15	0.2548	0.0482	-0.1057
temp1	26	0.2541	0.0248	<b>25</b>	<b>0.2741</b>	<b>0.0768</b>	0.0200
temp2	45	0.1793	0.0425	<b>48</b>	<b>0.2051</b>	<b>0.0430</b>	0.0257
temp3	20	0.3360	0.0224	<b>20</b>	<b>0.2618</b>	<b>0.0830</b>	<b>-0.0741</b>
<i>All</i>	91	0.1159	0.0274	93	0.1257	0.0330	0.0098

**Table S18.** Summary statistics and test significance for RS-based population load estimates (PopRS load) in wild emmer wheat samples collected in 1980 and in 2008. Kruskal–Wallis one-way ANOVA tests was performed with respect to population, group and year. A significance test (if found at 0.05, 0.01 or 0.001) is highlighted in blue, purple or red, respectively.

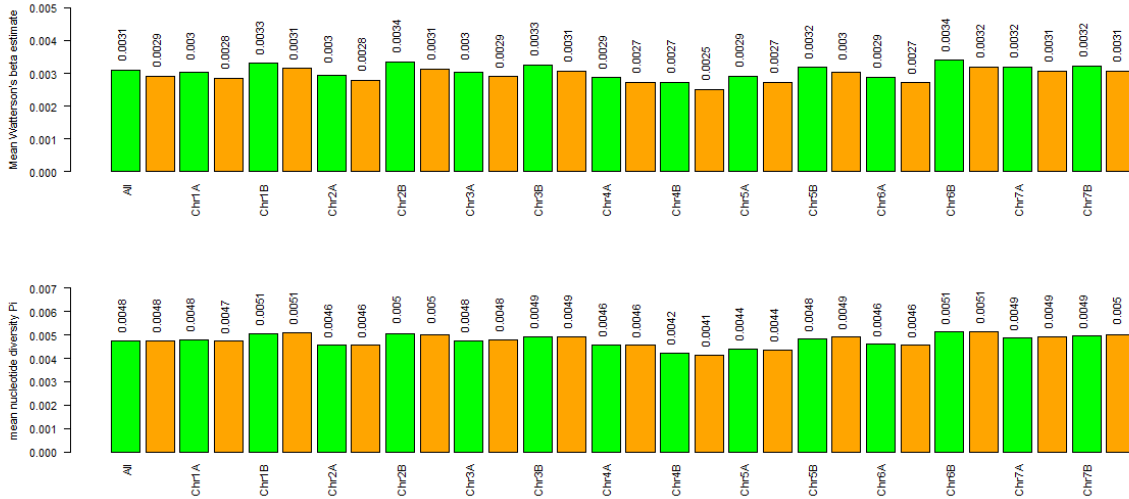
	Sample size	PopRS load	STD (PopRS load)	Sample size	PopRS load	STD (PopRS load)	Difference
	1980			2008			
<i>Population</i>							
1	7	0.783	0.789	6	0.656	0.633	-0.128
2	9	0.924	0.877	10	0.963	0.900	0.039
3	10	0.904	0.906	9	0.523	0.598	-0.380
4	10	0.679	0.761	10	1.054	0.985	0.375
5	10	0.816	0.799	10	0.718	0.771	-0.098
6	10	0.670	0.712	10	0.729	0.676	0.059
7	10	0.965	0.915	10	1.204	1.031	0.238
8	7	0.878	0.871	10	0.937	0.883	0.059
9	8	0.748	0.770	9	0.801	0.818	0.052
10	10	0.664	0.737	9	1.071	0.953	0.407
mean		0.803	0.814		0.866	0.825	0.062
<i>Group</i>							
rain1	45	0.327	0.428	49	0.366	0.474	0.039
rain2	29	0.377	0.486	29	0.497	0.546	0.120
rain3	17	0.586	0.693	15	0.411	0.505	-0.175
temp1	26	0.453	0.601	25	0.481	0.523	0.028
temp2	45	0.352	0.477	48	0.389	0.520	0.038
temp3	20	0.564	0.651	20	0.461	0.496	-0.103
<i>All</i>	91	0.235	0.380	93	0.258	0.388	0.023

## E. Figures S1 to S21

**Fig. S1.** A flowchart showing the overall procedures with eight major steps from population sampling (A1) to ontological analysis of deleterious genes (A8).

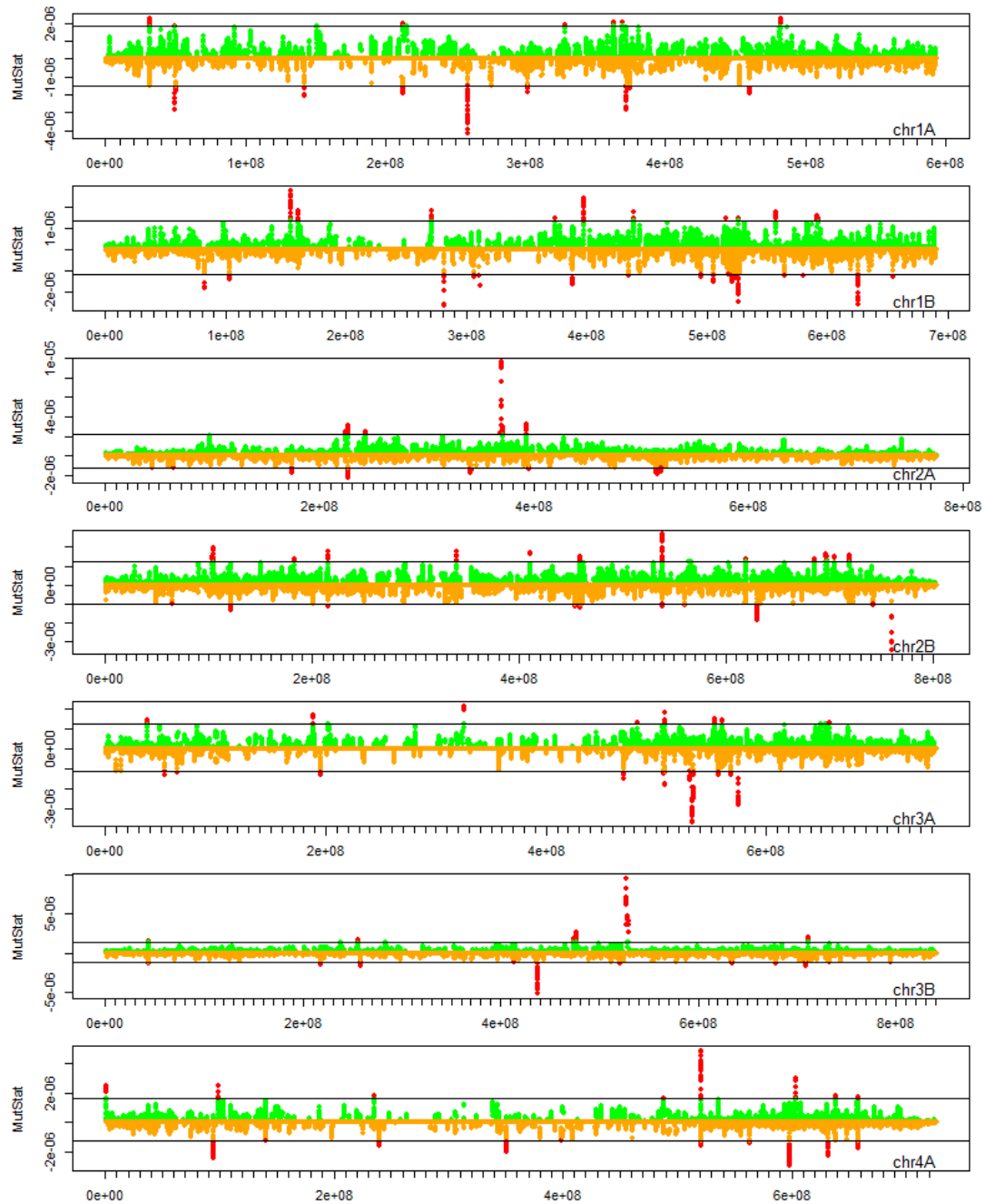


**Fig. S2.** Nucleotide diversity estimated with Watterson's Theta  $\theta$  (top panel) and Tajima's Pi  $\pi$  (bottom panel) across 14 chromosomes in individual samples of wild emmer wheat collected in 1980 and in 2008. These estimates were obtained across non-overlapping sliding windows of size 50,000 bp and step of 10,000 bp. The 1980 and 2008 sample groups were presented in green and orange bars, respectively. Overall, nucleotide diversity was lower in the 2008, than 1980, samples.

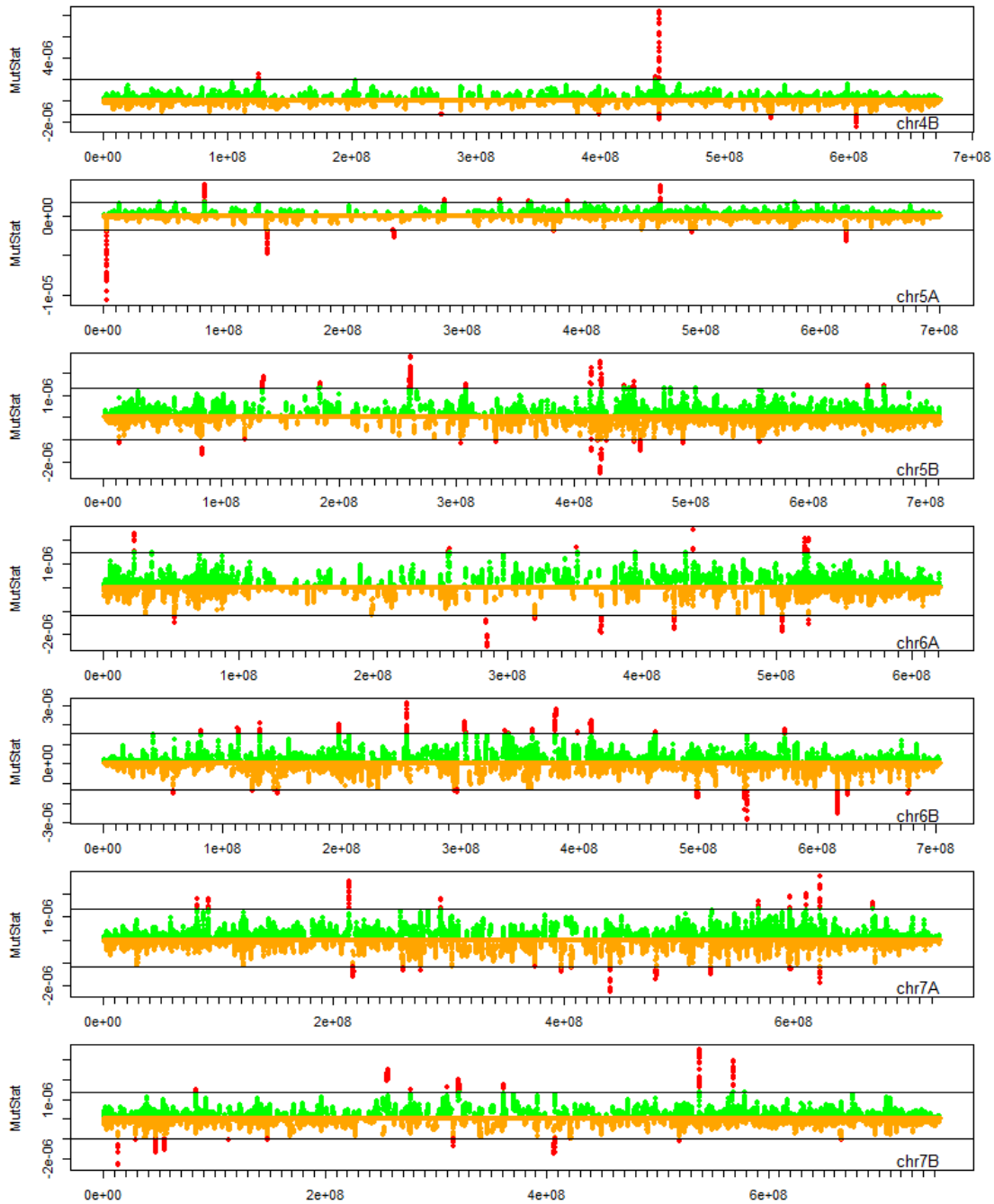




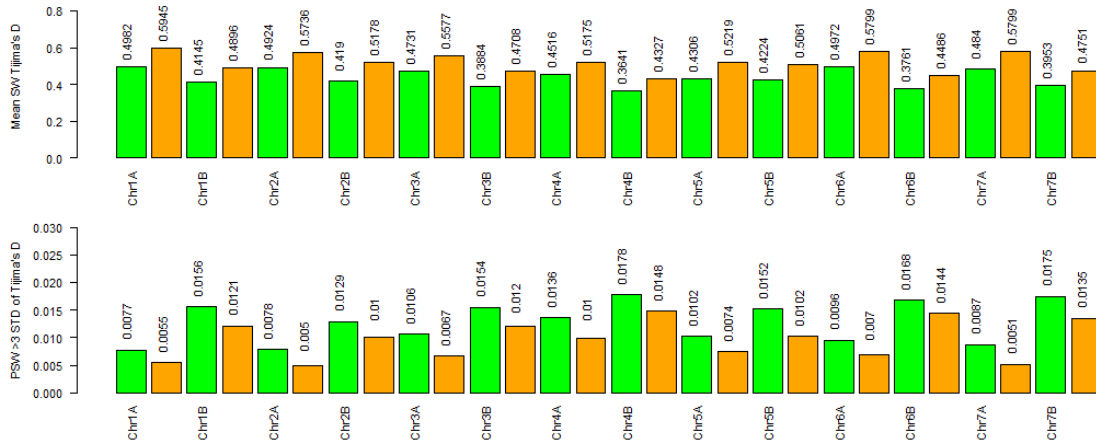
**Fig. S3a.** Selective sweeps across the first 7 wild emmer wheat chromosomes (chr1A to chr4A) identified by RAI<sub>SD</sub> mu statistics (MuStat) in the samples of wild emmer wheat collected in 1980 and in 2008. In each panel, upper and lower plots show MuStat for 1980 and 2008 samples for a chromosome, respectively, along with the highlight in red for those estimates greater than 15 standard deviations. Note each tick on a chromosome scale represents 10 million base pairs. Overall, more selective sweeps were identified in the 2008, than 1980, samples.



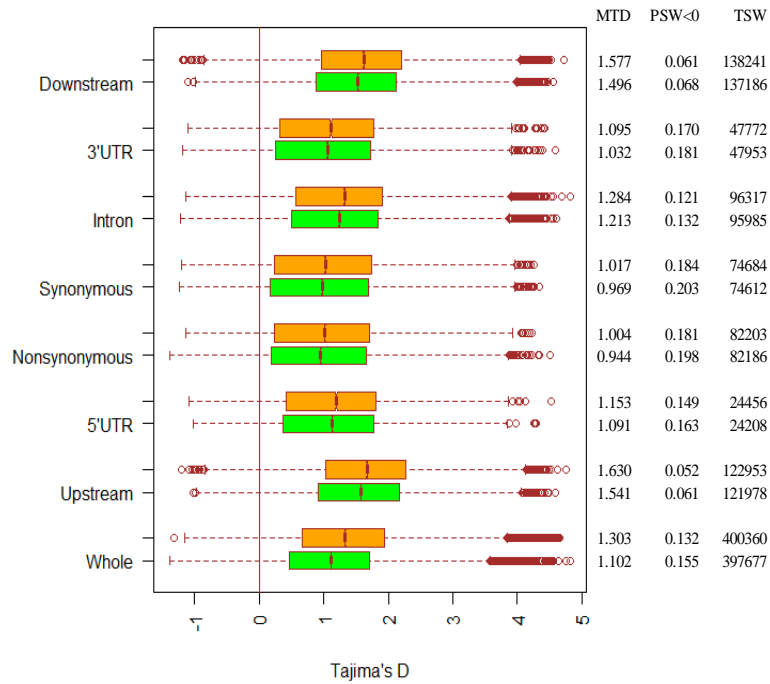
**Fig. S3b.** Selective sweeps across the second 7 wild emmer wheat chromosomes (chr4B to chr7B) identified by RAI<sub>SD</sub> mu statistics (MuStat) in the samples of wild emmer wheat collected in 1980 and in 2008. In each panel, upper and lower plots show MuStat for 1980 and 2008 samples for a chromosome, respectively, along with the highlight in red for those MuStats greater than 15 standard deviations. Note each tick on a chromosome scale represents 10 million base pairs. Overall, more selective sweeps were identified in the 2008, than 1980, samples.



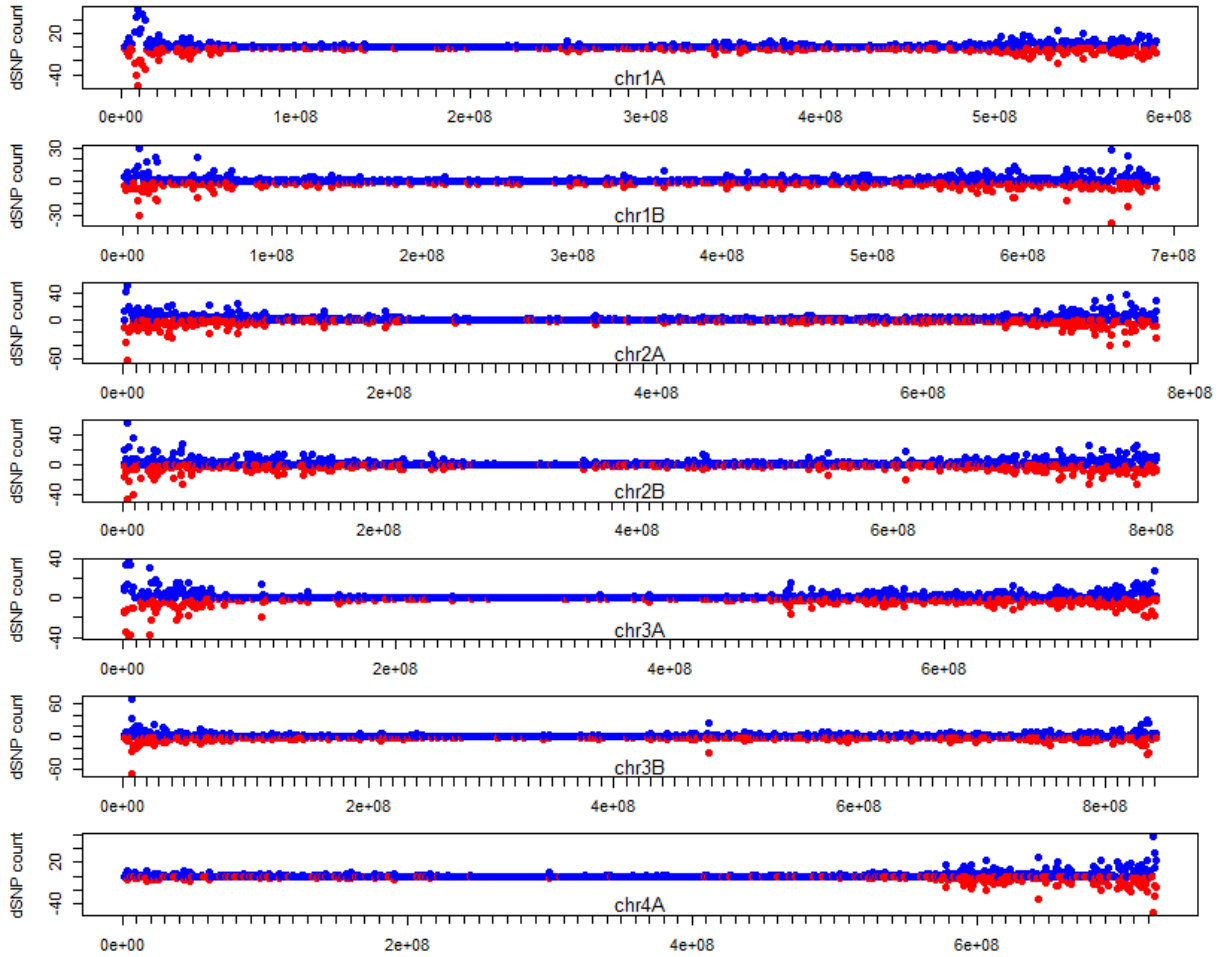
**Fig. S4.** Tajima's D statistics estimated across 14 chromosomes in the wild emmer wheat samples collected in 1980 and in 2008. In each panel, the green bar is for 1980 sample group, while the orange bar is for 2008 sample group. Mean values are presented above the bars. SW=sliding-window and PSW=the proportion of sliding-windows. The average Tajima's D statistics were higher in the 2008, than 1980, samples, and the proportional sliding-windows with Tajima's D statistics greater than 3 standard deviation were lower in the 2008, than 1980, samples.



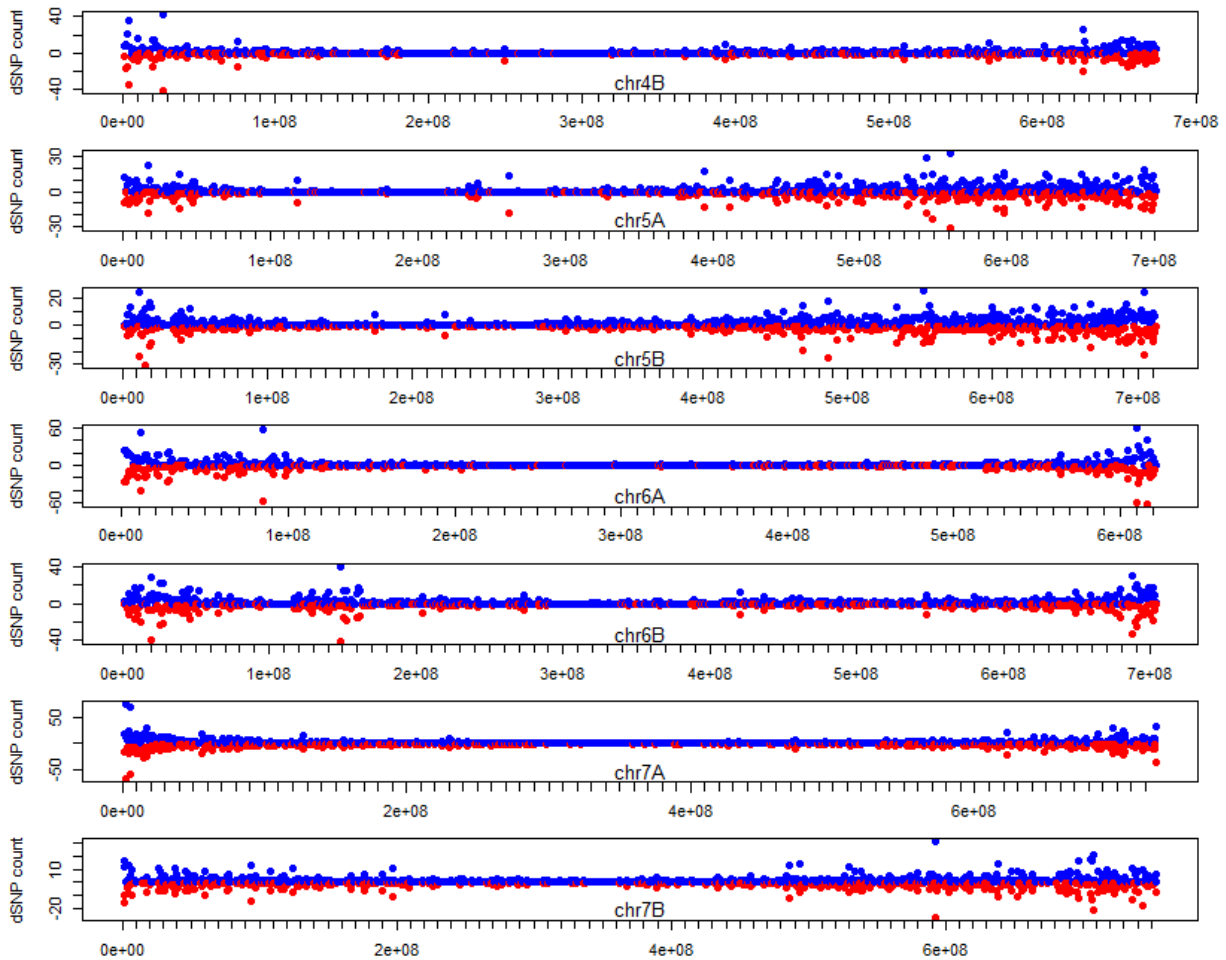
**Fig. S5.** Tajima's D statistics obtained in the samples of wild emmer wheat collected in 1980 and in 2008, with respect to different classes of sequence. Seven classes of sequence and whole genome were examined. For each class, the mean Tajima's D (MTD), the proportion of sliding windows with Tajima's D estimates smaller than zero (PSW<0), and total number of sliding windows (TSW) were also presented. The 1989 and 2008 sample groups were presented in green and orange bars, respectively. The proportions of sliding windows with Tajima's D estimates smaller than zero (PSW<0) were higher in the 2008, than 1980, samples for each class of sequence, implying the presence of more purging selection in the 2008 sample.



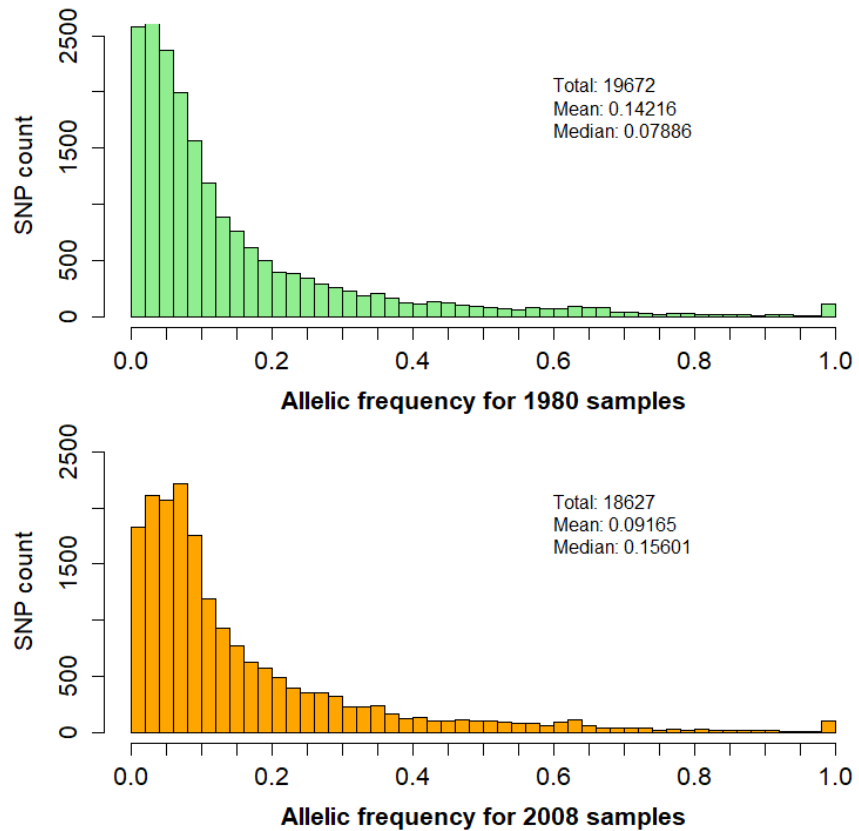
**Fig. S6a.** The distribution of deleterious SNPs (dSNPs) with non-overlapping sliding windows of 1 million base pairs across the first 7 chromosomes (Chr1A-Chr4A) for the 1980 and 2008 WEW samples, highlighted in blue and red circles, respectively. More dSNPs were distributed toward both ends of a chromosome, and such distribution patterns were similar for both 1980 and 2008 samples. Note each tick on a chromosome scale represents 10 million base pairs.



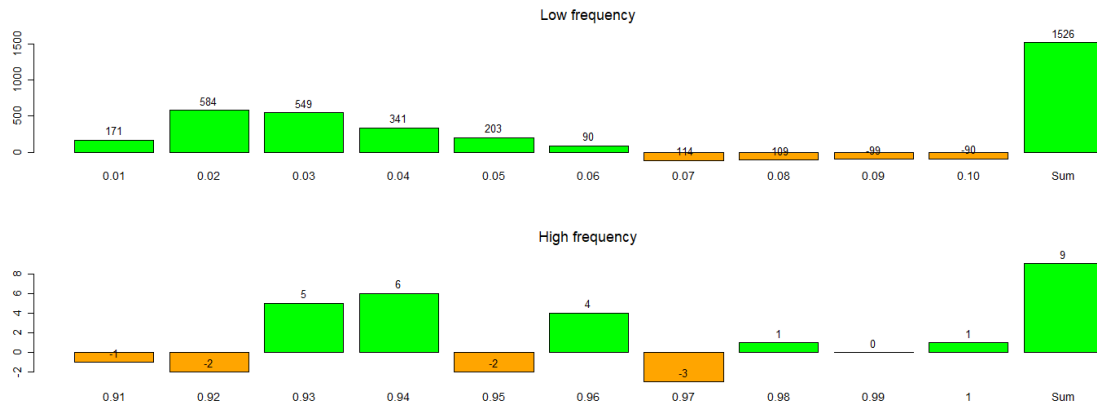
**Fig. S6b.** The distribution of deleterious SNPs (dSNPs) with non-overlapping sliding windows of 1 million base pairs across the second 7 chromosomes (Chr4B-Chr7B) for the 1980 and 2008 WEW samples, highlighted in blue and red circles, respectively. More dSNPs were distributed toward both ends of a chromosome, and such distribution patterns were similar for both 1980 and 2008 samples. Note each tick on a chromosome scale represents 10 million base pairs.



**Fig. S7.** Allele frequency (AF) distributions for deleterious SNPs (dSNPs) identified based on GERP++ RS scores determined from 12 plant species in individual samples of wild emmer wheat collected in 1980 and in 2008. In each panel, total dSNPs, mean AF and median AF are also shown. A majority of dSNPs had low allelic frequency, and the mean allelic frequency was lower in the 2008, than 1980, samples.

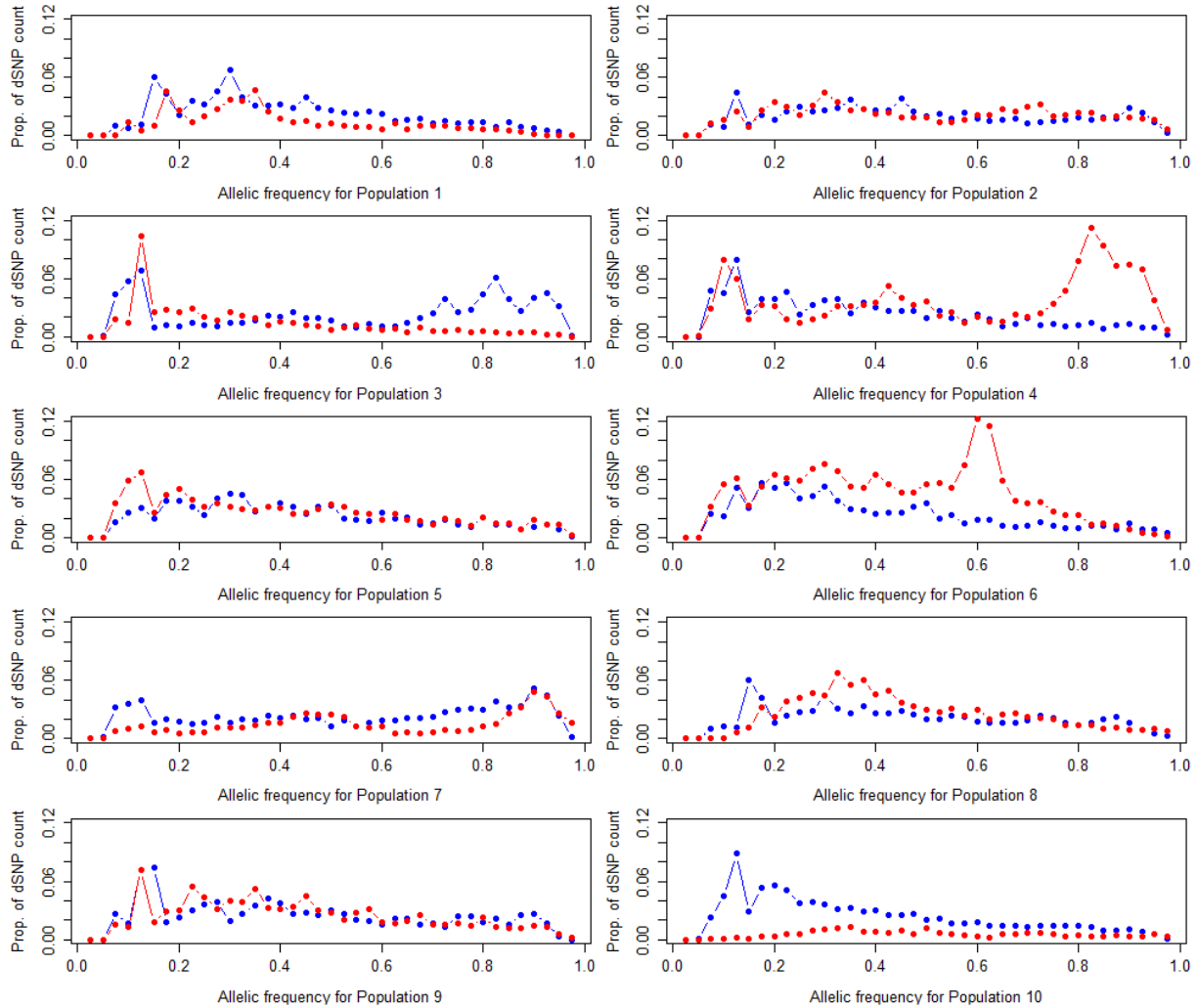


**Fig. S8.** Comparative counts of deleterious SNPs (dSNPs) with two extreme allelic frequencies in the samples of wild emmer wheat collected in 1980 and in 2008. Positive (in green) or negative (in orange) SNP count means more or fewer dSNPs in the 1980, than 2008, samples, respectively, with respect to extreme frequency range. A count difference is also shown for each bar. Sum means a difference in a total number of dSNPs with those allelic frequencies in each sample group. Overall, the 1980 sample had more dSNPs in the low and high frequency spectrums than the 2008 sample.

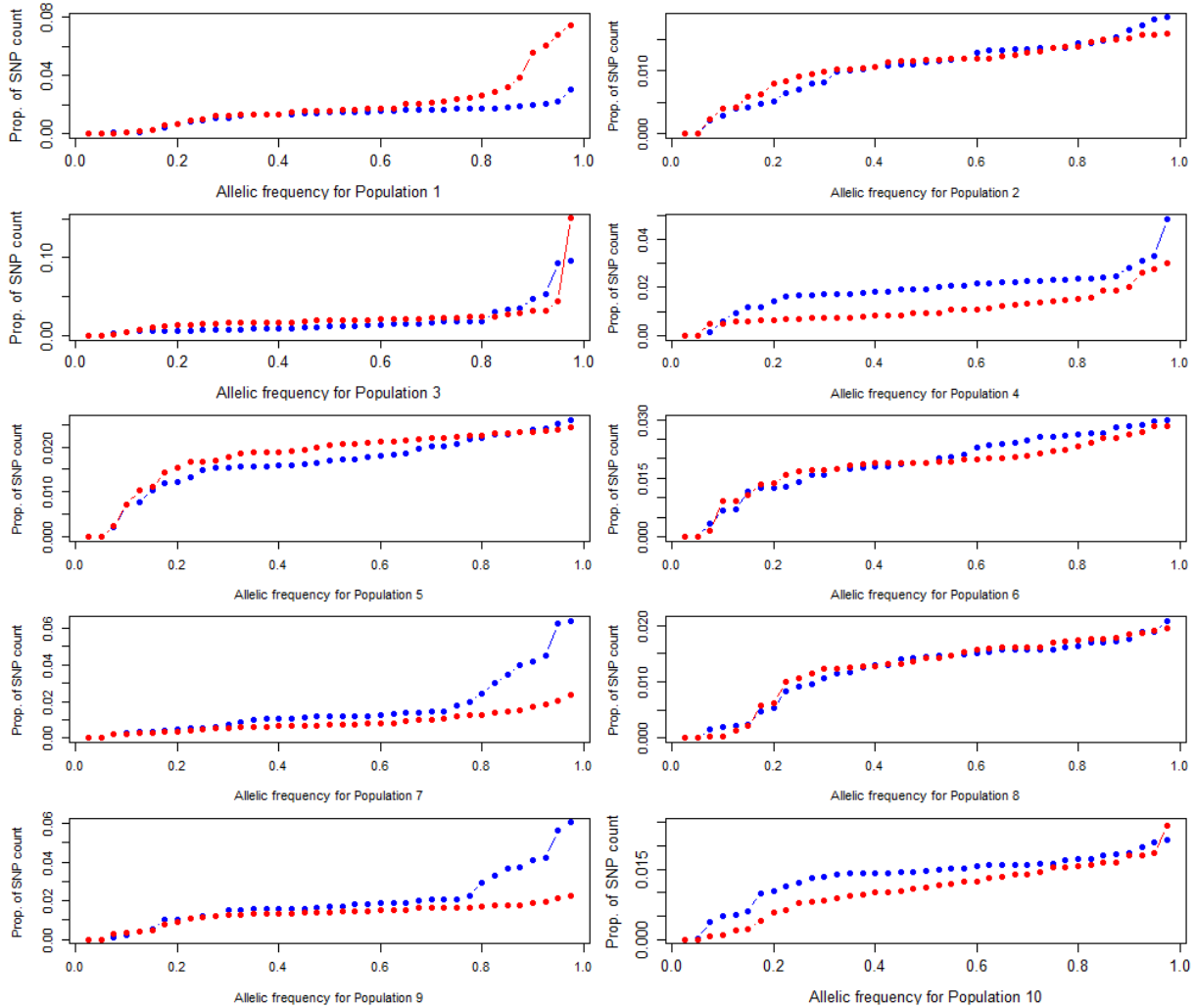




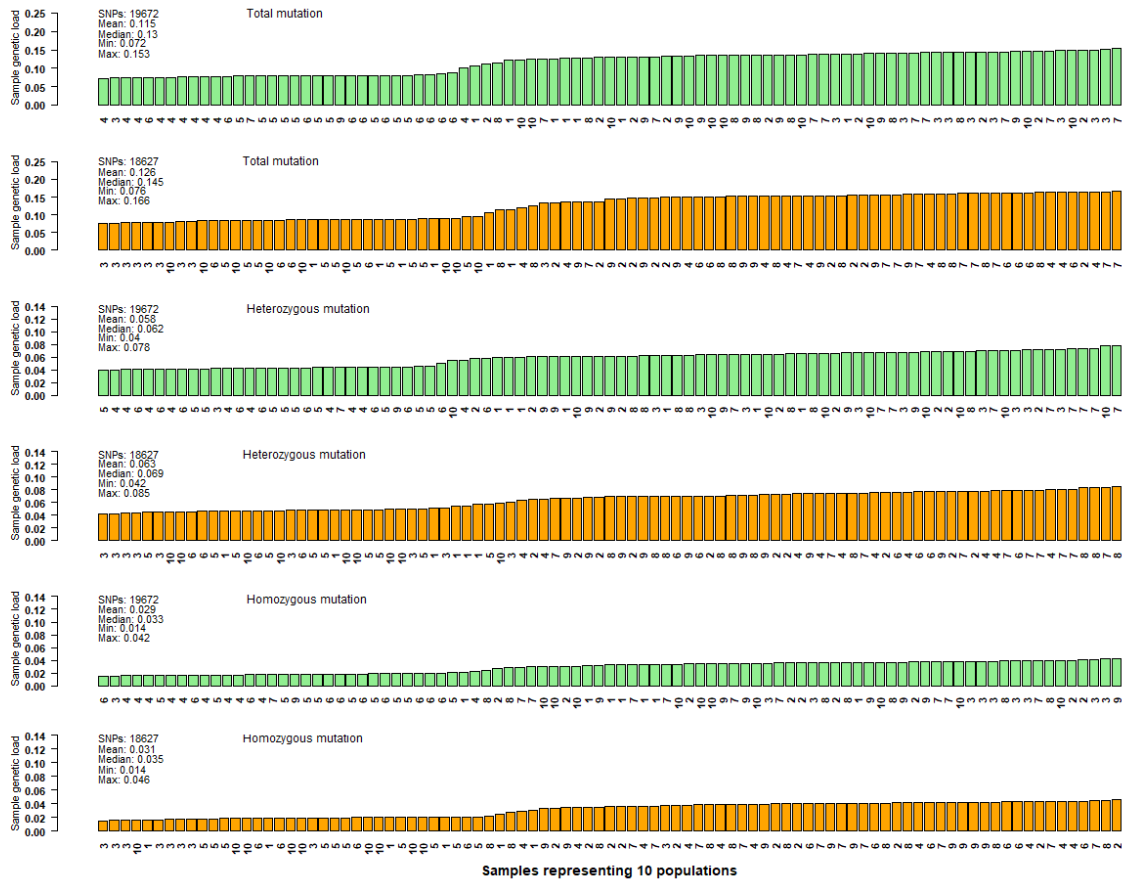
**Fig. S9.** Comparative allele frequency distributions for all the deleterious SNPs (dSNPs) detected in each population of wild emmer wheat between the collections in 1980 and in 2008. The frequency was highlighted in blue for the population samples collected in 1980 and in red for those in 2008. Marked allele frequency differences for all dSNPs were observed between the 1980 and 2008 samples collected in populations #1, 3, 4, 6, 7 and 10.



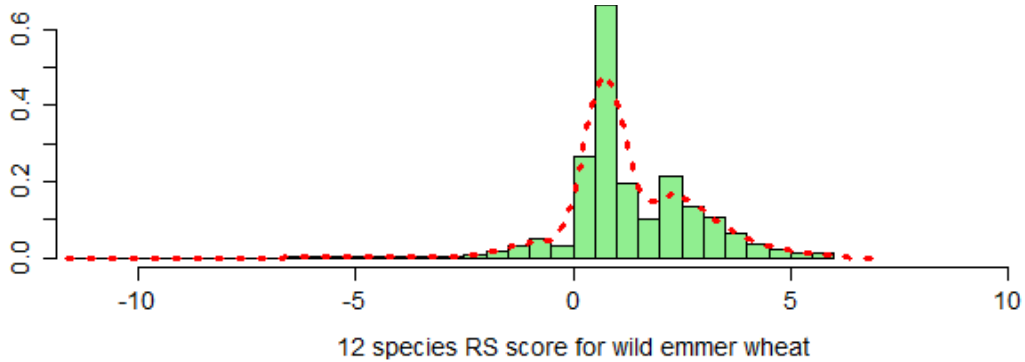
**Fig. S10.** Comparative allele frequency distributions for all the detected SNPs that were shared in each population of wild emmer wheat between the collections in 1980 and in 2008. The frequency was highlighted in blue for the population samples collected in 1980 and in red for those in 2008. Marked allele frequency differences for all detected SNPs were observed between the 1980 and 2008 samples collected in populations # 1, 4, 5, 7, 9 and 10.



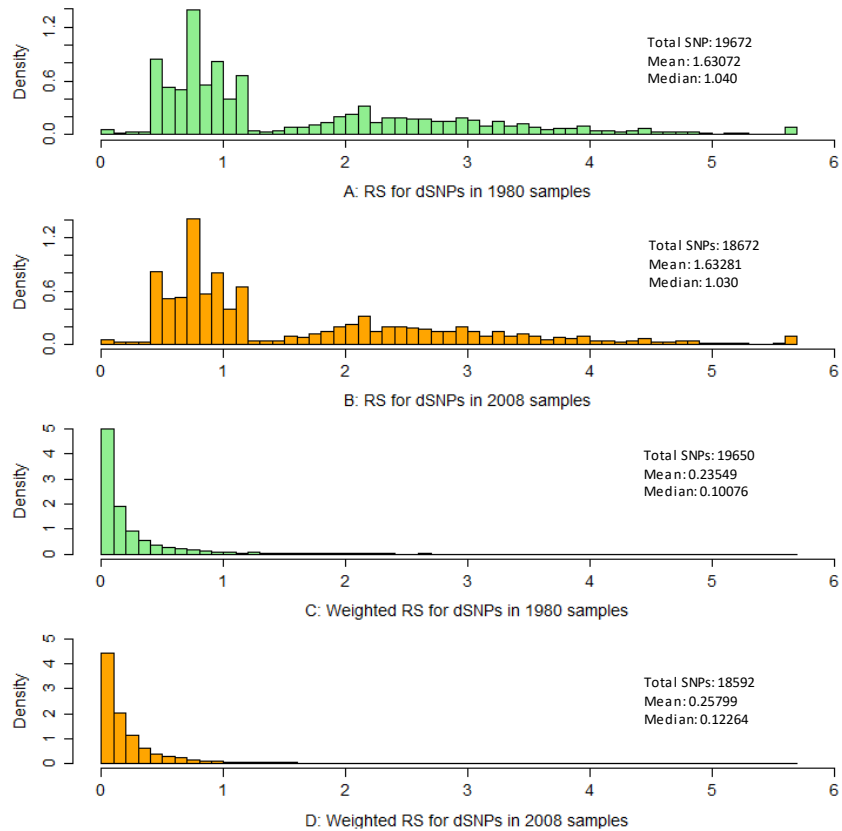
**Fig. S11.** Mutational burdens estimated for individual samples of wild emmer wheat collected in 1980 and in 2008. Three mutational burdens were presented in total mutation, heterozygous mutation and homozygous mutation. Three green panels are for the 1980 sample and three orange panels for the 2008 sample. Each panel also shows the total deleterious SNPs and other statistics for the mutational burdens and each sample was labeled according to its population numbering from the northern to southern Israel region. Overall, these individual mutational burdens were not associated with their population latitudes.



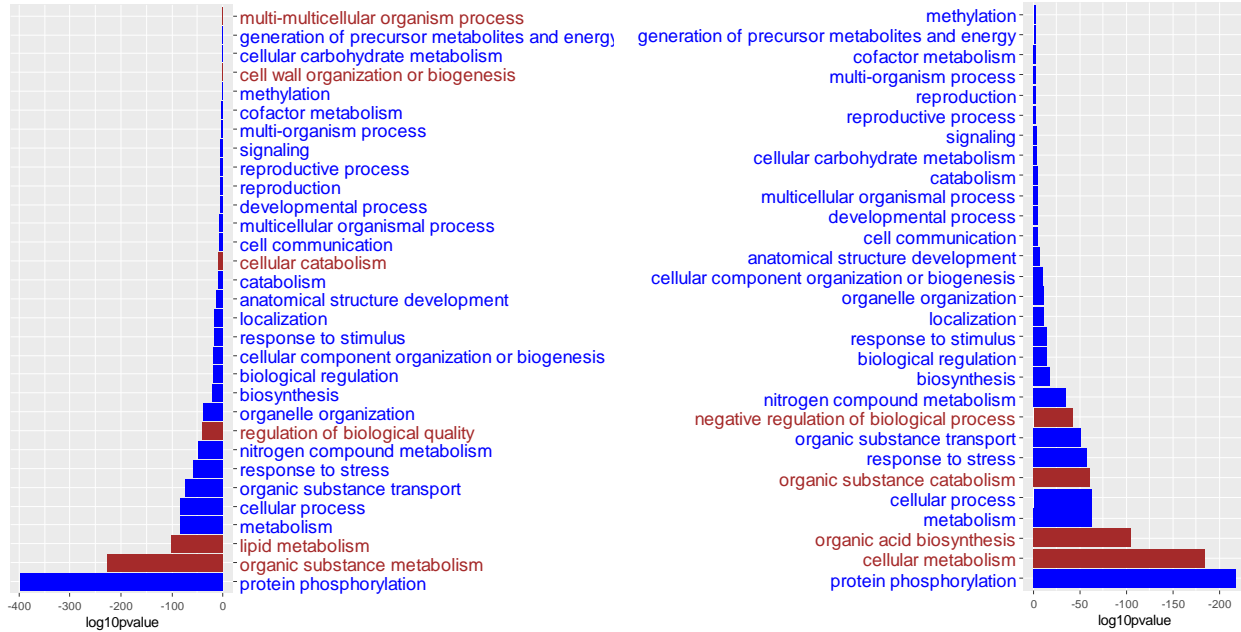
**Fig. S12.** Distribution of GERP++ RS scores based on 12 species for SNPs identified from exome capture data in the combined samples of wild emmer wheat collected in 1980 and in 2008.



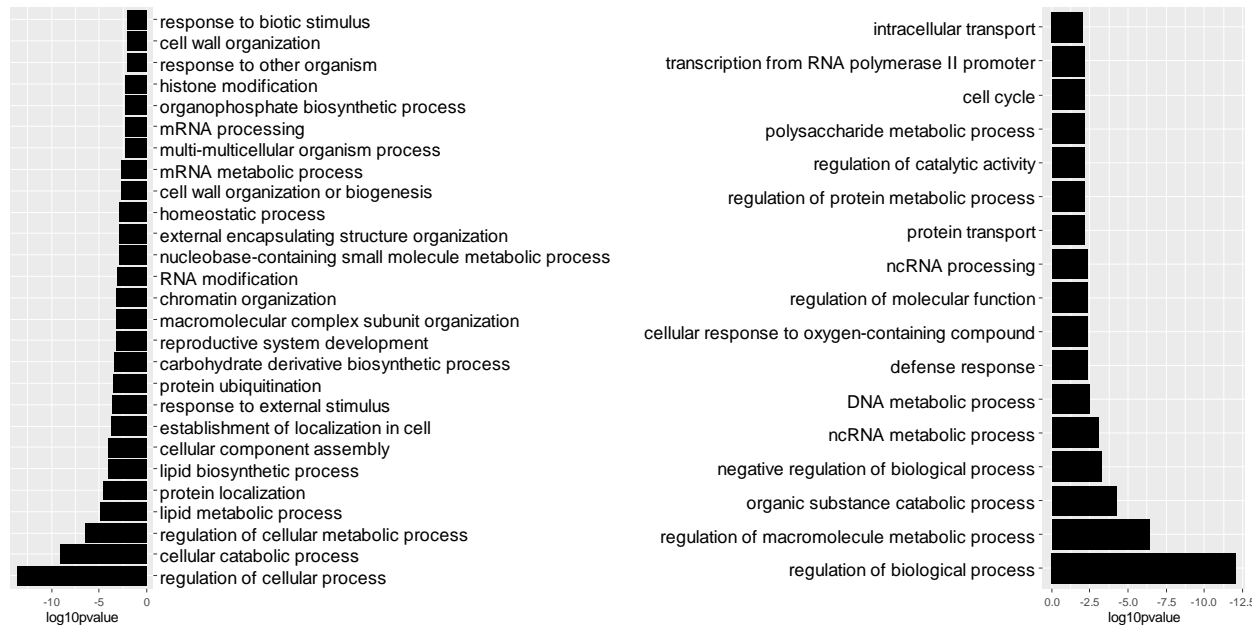
**Fig. S13.** Distributions of RS and weighted RS scores for deleterious SNPs (dSNPs) in the 1980 and 2008 wild emmer wheat samples. The RS distributions were similar for dSNPs detected in both 1980 and 2008 samples, but the mean value of weighted RS scores was larger in the 2008, than 1980, samples.



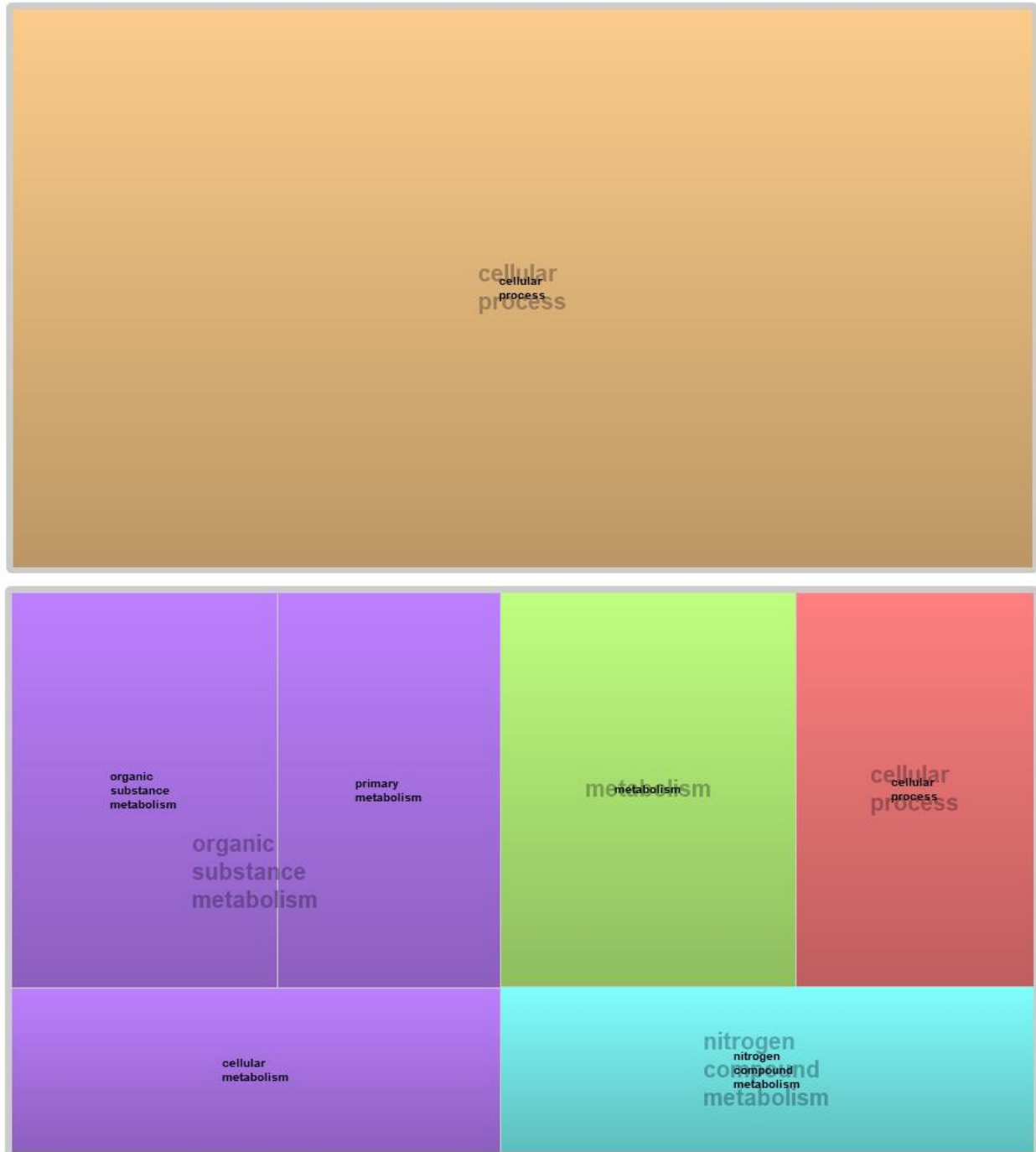
**Fig. S14a.** REVIGO gene ontology cluster representations showing the shared (in blue) and unique (in brown) biological processes associated with 317 and 272 GO terms extracted from 9,616 and 9,091 deleterious genes that were unique to the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively. More unique biological processes were observed in the 1980, than 2008, samples.



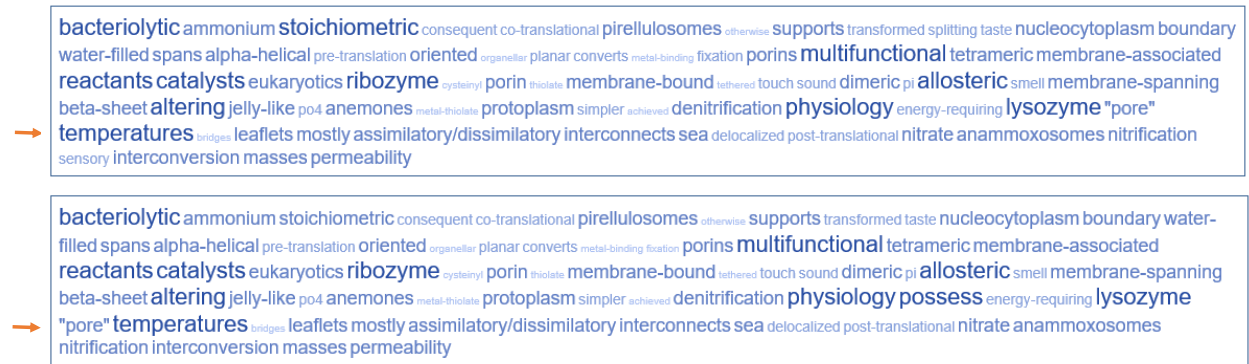
**Fig. S14b.** An illustration of 28 and 18 unique REVIGO gene ontology biological processes associated with 317 and 272 GO terms extracted from 9,616 and 9,091 deleterious genes that were unique to the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively.



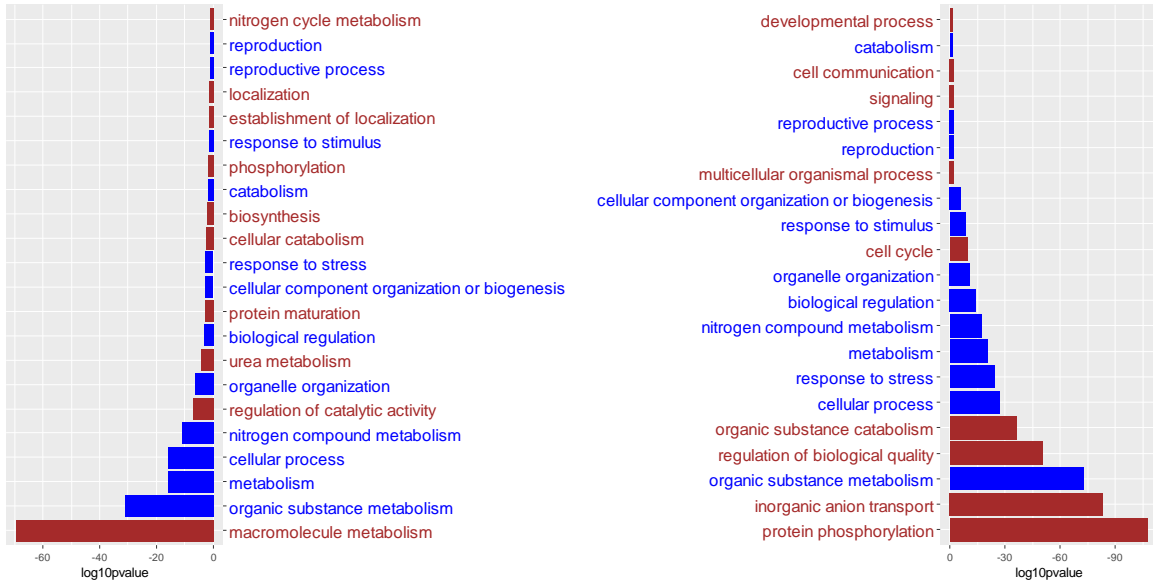
**Fig. S15.** REVIGO gene ontology treemaps showing cluster representatives of the biological processes associated with 3 and 14 GO terms extracted from 93 and 93 fixed deleterious genes that were unique to the samples of wild emmer wheat collected in 1980 (top panel) or in 2008 (bottom panel), respectively. More representative biological processes observed in the 2008, than 1980, samples imply that the fixation of deleterious genes was widely spread into various biological processes in the 2008 samples.



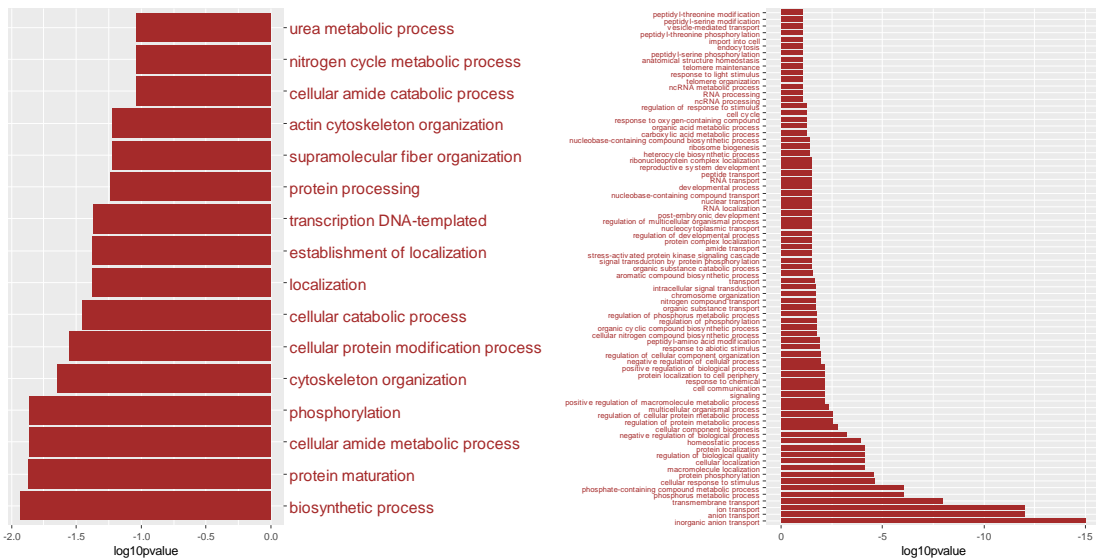
**Fig. S16.** Tag clouds showing keywords that correlate with the values based on 1,044 and 1,022 GO terms for all the deleterious genes detected in the samples of wild emmer wheat collected in 1980 (top panel) and in 2008 (bottom panel), respectively. Each panel shows the keywords associated with the assayed GO terms for all deleterious genes, including a word of *temperature*, as highlighted in red arrow on the left, implying some of the assayed deleterious genes were associated with climate factor temperature.



**Fig. S17a.** An illustration of the shared (in blue) and unique (in brown) REVIGO gene ontology cluster representations for biological processes associated with 159 and 336 GO terms extracted from 66 and 80 non-redundant genes that were identified by RAiSD MuStat estimates of 20 standard deviations in the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively. More genes were under-represented with smaller log10pvalue in the 2008, than 1980, samples.

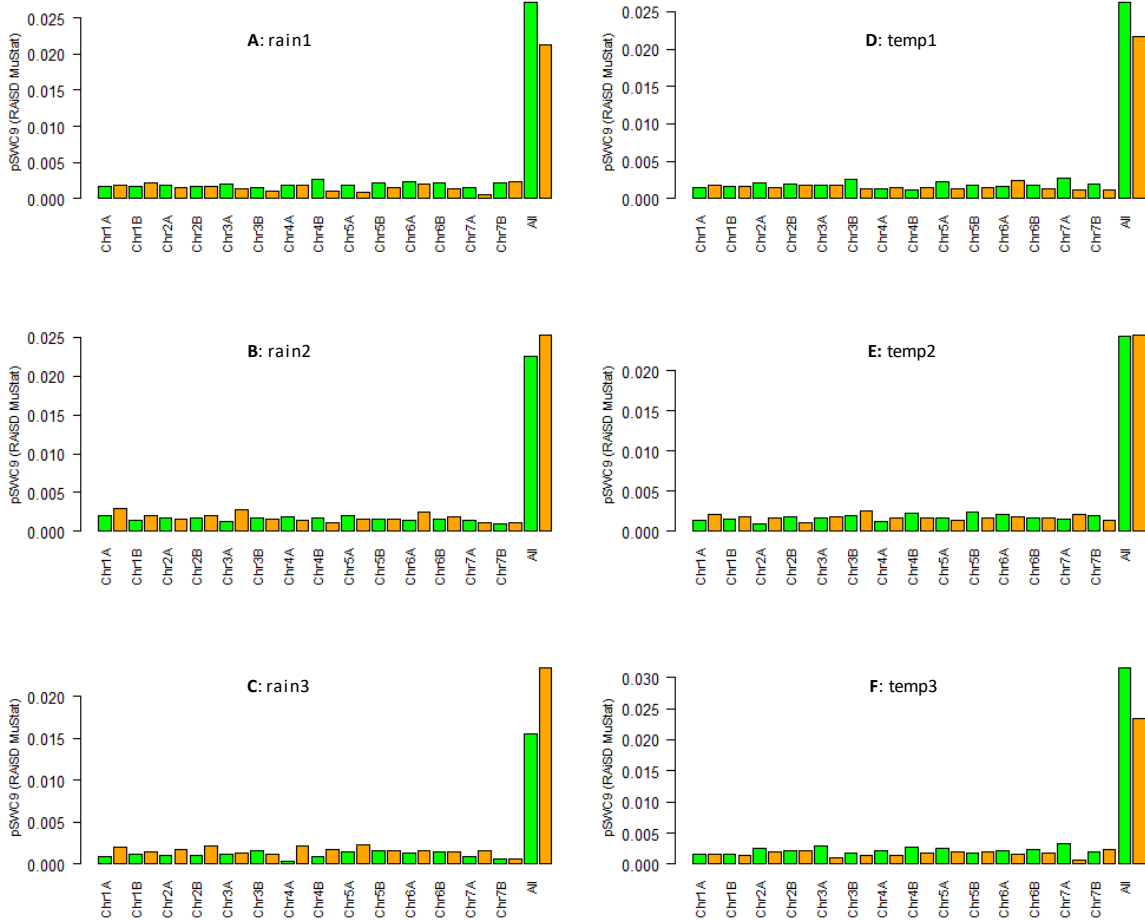


**Fig. S17b.** An illustration of 16 and 77 unique REVIGO gene ontology biological processes associated with 159 and 336 GO terms extracted from 66 and 80 non-redundant genes that were identified by RAiSD MuStat estimates of 20 standard deviations in the samples of wild emmer wheat collected in 1980 (the left panel) or in 2008 (the right panel), respectively. More unique biology processes in the 2008, than 1980, samples indicate the presence of more selective genes in the chromosomal regions identified by RAiSD.

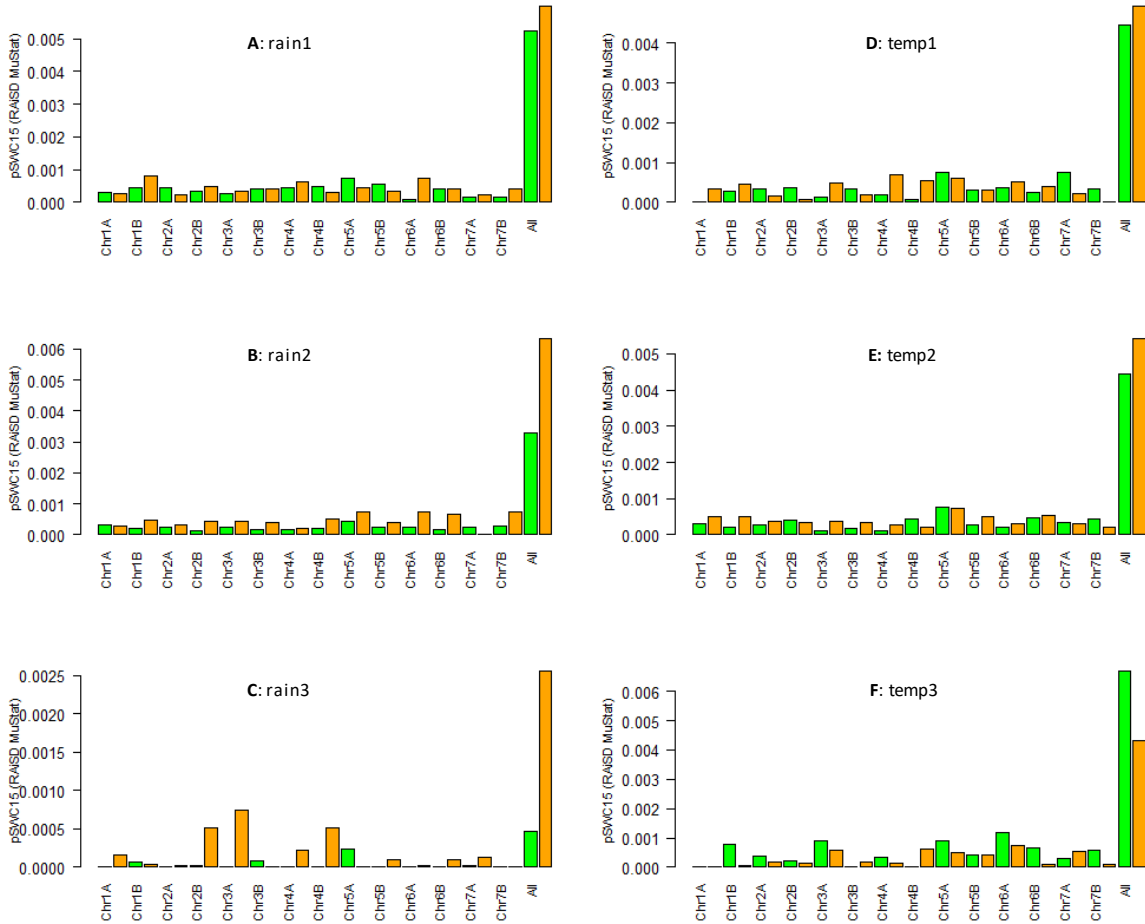




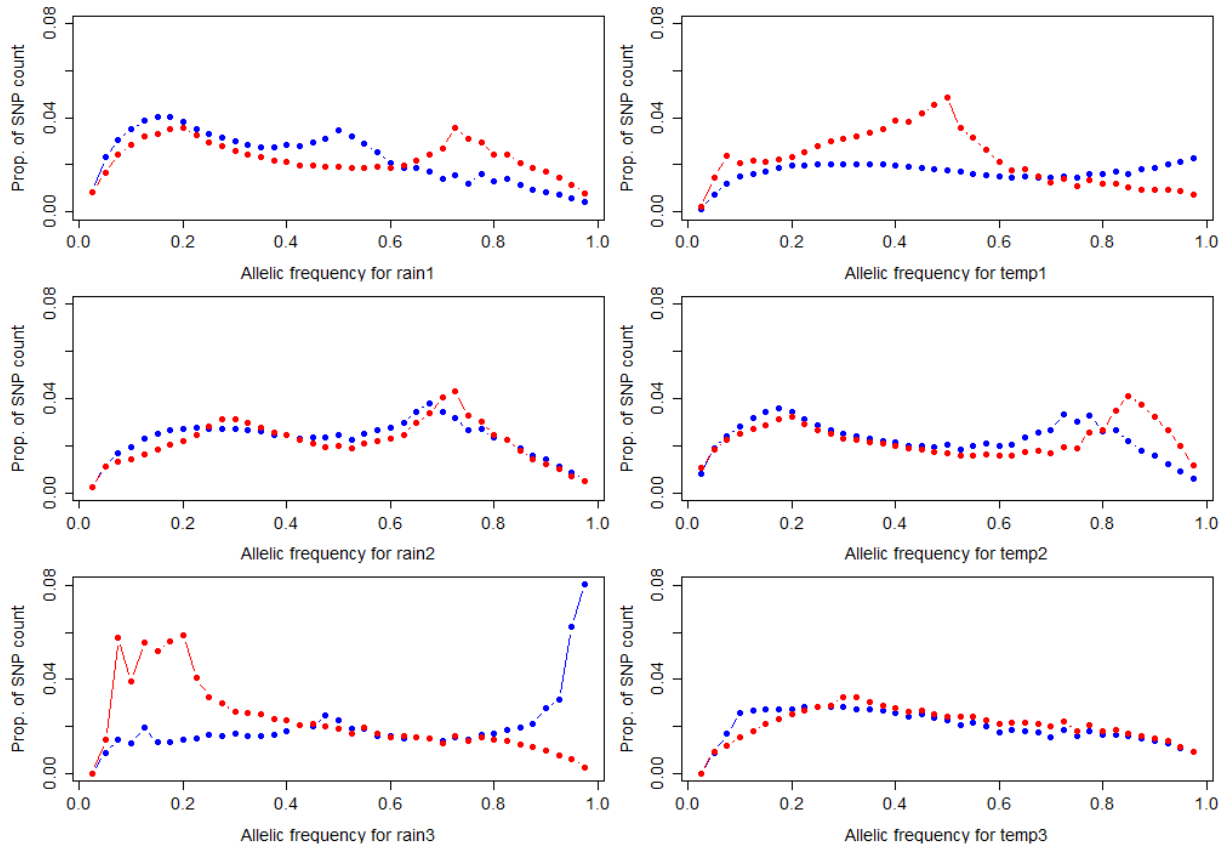
**Fig. S18.** The proportions of significant sliding windows per chromosome with 9 standard deviations of RAiSD MuStat (pSWC9) in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008. The 1980 and 2008 sample groups are labelled in green and orange, respectively. These results show that the selection signals varied among climate-specific groups.



**Fig. S19.** The proportions of significant sliding windows per chromosome with 15 standard deviations of RAiSD MuStat (pSWC15) in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008. The 1980 and 2008 sample groups are labelled in green and orange, respectively. At pSWC15, more selective sweeps were identified in the 2008, than 1980, samples for five climate-specific groups, except for temp3.



**Fig. S20.** Comparative allele frequency distributions for all the SNPs detected in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008. The frequency was highlighted in blue for the wild emmer samples collected in 1980 and in red for those in 2008. Marked allele frequency differences for all detected SNPs were observed between the 1980 and 2008 samples collected in the climate-specific groups of rain1, rain3, temp1 and temp2.



**Fig. S21.** Comparative allele frequency distributions for all the deleterious SNPs (dSNPs) detected in six climate-specific groups of wild emmer wheat samples collected in 1980 and in 2008. The frequency was highlighted in blue for the wild emmer samples collected in 1980 and in red for those in 2008. Marked allele frequency differences for dSNPs were observed between the 1980 and 2008 samples collected in the climate-specific groups of rain2, rain3, temp1 and temp3.

